Express Mail Certificate No. EU212806766US
Date Deposited: August 28, 2003

POLYNUCLEOTIDES AND POLYPEPTIDES, MATERIALS INCORPORATING THEM AND METHODS FOR USING THEM

Cross-Reference to Related Applications

This application claims priority to U.S. Patent Application No. 60/406,810, filed August 28, 2002.

Technical Field of the Invention

This invention relates to polynucleotides isolated from lactic acid bacteria as well as to probes and primers specific to the polynucleotides; genetic constructs comprising the polynucleotides; biological materials, including plants, microorganisms and multicellular organisms, incorporating the polynucleotides; polypeptides expressed by the polynucleotides; and methods for using the polynucleotides and polypeptides.

Background of the Invention

The present invention relates to polynucleotides isolated from a specific strain of lactic acid bacteria, namely *Lactobacillus rhamnosus* HN001 (*L. rhamnosus* HN001). Lactic acid bacteria, and their enzymes, are the major determinants of flavor and fermentation characteristics in fermented dairy products, such as cheese and yogurt. Flavors are produced through the action of bacteria and their enzymes on proteins, carbohydrates and lipids.

Lactobacillus rhamnosus strain HN001 are heterofermentative bacteria that are Gram positive, non-motile, non-spore forming, catalase negative, facultative anaerobic rods exhibiting

an optimal growth temperature of 37±1°C and an optimum pH of 6.0 – 6.5. Experimental studies demonstrated that dietary supplementation with *Lactobacillus rhamnosus* strain HN001 induced a sustained enhancement in several aspects of both natural and acquired immunity (*See* PCT International Publication No. WO 99/10476). In addition, *L. rhamnosus* HN001, and certain other Gram-positive bacteria can specifically and directly modulate human and animal health (See, for example, Tannock *et al.*, *Applied Environ. Microbiol.* 66:2578-2588, 2000; Gill *et al.*, *Brit. J. Nutrition* 83:167-176; Quan Shu *et al.*, *Food and Chem. Toxicol.* 38:153-161, 2000; Quan Shu *et al.*, *Intl. J. Food Microbiol.* 56:87-96, 2000; Quan Shu *et al.*, *Intl. Dairy J.* 9:831-836, 1999; Prasad *et al.*, *Intl. Dairy J.* 8:993-1002, 1998; Sanders and Huis in't Veld, *Antonie van Leeuwenhoek* 76:293-315, 1999; Salminen *et al.*, 1998. *In*: Lactic Acid Bacteria, Salminen S and von Wright A (eds)., Marcel Dekker Inc, New York, Basel, Hong Kong, pp. 211-253; Delcour *et al.*, *Antonie van Leeuwenhoek* 76:159-184, 1999; Blum *et al.*, *Antonie van Leeuwenhoek* 76:199-205, 1999; Yasui *et al.*, *Antonie van Leeuwenhoek* 76:383-389, 1999; Hirayama and Rafter, *Antonie van Leeuwenhoek* 76:391-394, 1999; Ouwehand, 1998. *In*: Lactic Acid Bacteria, Salminen S and von Wright A (eds)., Marcel Dekker Inc, New York, Basel, Hong Kong, pp. 139-159; Isolauri *et al.*, S 1998. *In*: Lactic Acid Bacteria, Salminen S and von Wright A (eds)., Marcel Dekker Inc, New York, Basel, Hong Kong, pp. 255-268; Lichtenstein and Goldin, 1998. *In*: Lactic Acid Bacteria, Salminen S and von Wright A (eds)., Marcel Dekker Inc, New York, Basel, Hong Kong, pp. 269-277; El-Nezami and Ahokas, 1998. *In*: Lactic Acid Bacteria, Salminen S and von Wright A (eds)., Marcel Dekker Inc, New York, Basel, Hong Kong, pp. 359-367; Nousianen *et al.*, 1998. *In*: Lactic Acid Bacteria, Salminen S and von Wright A (eds)., Marcel Dekker Inc, New York, Basel, Hong Kong, pp. 437-473; Meisel and Bockelmann, *Antonie van Leeuwenhoek* 76:207-215, 1999; Christensen *et al.*, *Antonie van Leeuwenhoek* 76:217-246, 1999; Dunne *et al.*, *Antonie van Leeuwenhoek* 76:279-292, 1999). Beneficial health effects attributed to these bacteria include the following:

Increased resistance to enteric pathogens and anti-infection activity, including treatment of rotavirus infection and infantile diarrhea – due to increases in antibody production caused by an adjuvant effect, increased resistance to pathogen colonization; alteration of intestinal

conditions, such as pH; and the presence of specific antibacterial substances, such as bacteriocins and organic acids.

Aid in lactose digestion – due to lactose degradation by bacterial lactase enzymes (such as beta-galactosidase) that act in the small intestine.

Anti-cancer (in particular anti-colon cancer) and anti-mutagenesis activities – due to anti-mutagenic activity; alteration of procancerous enzymatic activity of colonic microbes; reduction of the carcinogenic enzymes azoreductase, beta-glucuronidase and nitroreductase in the gut and/or faeces; stimulation of immune function; positive influence on bile salt concentration; and antioxidant effects.

Liver cancer reduction – due to aflatoxin detoxification and inhibition of mould growth.

Reduction of small bowel bacterial overgrowth – due to antibacterial activity; and decrease in toxic metabolite production from overgrowth flora.

Immune system modulation and treatment of autoimmune disorders and allergies – due to enhancement of non-specific and antigen-specific defence against infection and tumors; enhanced mucosal immunity; adjuvant effect in antigen-specific immune responses; and regulation of Th1/Th2 cells and production of cytokines.

Treatment of allergic responses to foods– due to prevention of antigen translocation into blood stream and modulation of allergenic factors in food.

Reduction of blood lipids and prevention of heart disease – due to assimilation of cholesterol by bacteria; hydrolysis of bile salts; and antioxidative effects.

Antihypertensive effect - bacterial protease or peptidase action on milk peptides produces antihypertensive peptides. Cell wall components act as ACE inhibitors

Prevention and treatment of urogenital infections – due to adhesion to urinary and vaginal tract cells resulting in competitive exclusion; and production of antibacterial substances (acids, hydrogen peroxide and biosurfactants).

Treatment of inflammatory bowel disorder and irritable bowel syndrome – due to immuno-modulation; increased resistance to pathogen colonization; alteration of intestinal conditions such as pH; production of specific antibacterial substances such as bacteriocins, organic acids and hydrogen peroxide and biosurfactants; and competitive exclusion.

Modulation of infective endocarditis – due to fibronectin receptor-mediated platelet aggregation associated with *Lactobacillus* sepsis.

Prevention and treatment of *Helicobacter pylori* infection – due to competitive colonization and antibacterial effect.

Prevention and treatment of hepatic encephalopathy – due to inhibition and/or exclusion of urease-producing gut flora.

Improved protein and carbohydrate utilisation and conversion – due to production of beneficial products by bacterial action on proteins and carbohydrates.

Other beneficial health effects associated with *L. rhamnosus* include: improved nutrition; regulation of colonocyte proliferation and differentiation; improved lignan and isoflavone metabolism; reduced mucosal permeability; detoxification of carcinogens and other harmful compounds; relief of constipation and diarrhea; and vitamin synthesis, in particular folate.

Peptidases are enzymes that break the peptide bonds linking the amino group of one amino acid with the carboxy group (acid group) of an adjacent amino acid in a peptide chain. The bonds are broken in a hydrolytic reaction. There is a large family of peptidase enzymes that are defined by their specificity for the particular peptides bonds that they cleave (Barrett A J,

Rawlings N D and Woessner J F (Eds.) 1998. *Handbook of proteolytic enzymes*. Academic Press, London, UK). The two main families are exopeptidases and endopeptidases.

Exopeptidases cleave amino acids from the N- or C- terminus of a peptide chain, releasing free amino acids or short (di- and tri-) peptides. Different types of exopeptidases include:

- Aminopeptidases - release a free amino acid from the N-terminus of a peptide chain;
- dipeptidyl-peptidase (also known as dipeptidyl-aminopeptidases) - release a dipeptide from the N-terminus of a peptide chain;
- tripeptidyl-peptidases (also known as tripeptidyl-aminopeptidases) - release a tripeptide from the N-terminus of a peptide chain);
- carboxypeptidases - release a free amino acid from the C-terminus of a peptide chain;
- peptidyl-dipeptidase - release a dipeptide from the C-terminus of a peptide chain;
- dipeptidases - release two free amino acids from a dipeptide; and
- tripeptidases - release a free amino acid and a dipeptide from a tripeptide.

Peptidases are important enzymes in the process of cheese ripening and the development of cheese flavor. The hydrolysis of milk caseins in cheese results in textural changes and the development of cheese flavors. The raft of proteolytic enzymes that cause this hydrolysis come from the lactic acid bacteria that are bound up in the cheese – either starter cultures that grow up during the manufacture of the cheese, or adventitious and adjunct non-starter lactic acid bacteria that grow in the cheese as it ripens (Law Haandrikman, *Int. Dairy J.* 7:1-11, 1997).

Many other enzymes can also influence dairy product flavor, and functional and textural characteristics, as well as influencing the fermentation characteristics of the bacteria, such as speed of growth, acid production and survival (Urbach, *Int. Dairy J.* 5:877-890, 1995; Johnson and Somkuti, *Biotech. Appl. Biochem.* 13:196-204, 1991; El Soda and Pandian, *J. Dairy Sci.* 74:2317-2335, 1991; Fox *et al,*. In Cheese: chemistry, physics and microbiology. Volume 1, General aspects, 2nd edition, P Fox (ed) Chapman and Hall, London; Christensen *et al., Antonie van Leeuwenhoek* 76:217-246, 1999; Stingle *et al., J. Bacteriol.* 20:6354-6360, 1999; Stingle *et al., Mol. Microbiol.* 32:1287-1295, 1999; Lemoine *et al., Appl. Environ. Microbiol.* 63:1512-

3518, 1997). Enzymes influencing specific characteristics and/or functions include the following:

- **Lysis of cells.** These enzymes are mostly cell wall hydrolases, including amidases; muramidases; lysozymes, including N-acetyl muramidase; muramidase; N-acetylglucosaminidase; and N-acetylmuramoyl-L-alanine amidase. DEAD-box helicase proteins also influence autolysis.

- **Carbohydrate utilization.** Lactose, citrate and diacetyl metabolism, and alcohol metabolism are particularly important. The enzymes involved include beta-galactosidase, lactate dehydrogenase, citrate lyase, citrate permease, 2,3 butanediol dehydrogenase (acetoin reductase), acetolactate decarboxylase, acetolactate synthase, pyruvate decarboxylase, pyruvate formate lyase, diacetyl synthase, diacetyl reductase, alcohol decarboxylase, lactate dehydrogenase, pyruvate dehydrogenase, and aldehyde dehydrogenase.

- **Lipid degradation, modification or synthesis.** Enzymes involved include lipases, esterases, phospholipases, serine hydrolases, desaturases, and linoleate isomerase.

- **Polysaccharide synthesis.** Polysaccharides are important not only for potential immune enhancement and adhesion activity but are important for the texture of fermented dairy products. The enzymes involved are a series of glucosyl transferases, including beta-(1-3) glucosyl transferase, alpha-N acetylgalactosaminyl transferase, phosphogalactosyl transferase, alpha-glycosyl transferase, UDP-N-acetylglucosamine C4 epimerase and UDP-N-acetylglucosamine transferase.

- **Amino acid degradation.** Enzymes include glutamate dehydrogenase, aminotransferases, amino acid decarboxylases, and enzymes involved in sulphur amino acid degradation including cystathione beta-lyase.

Sequencing of the genomes, or portions of the genomes, of numerous organisms, including humans, animals, microorganisms and various plant varieties, has been and is being

carried out on a large scale. Polynucleotides identified using sequencing techniques may be partial or full-length genes, and may contain open reading frames, or portions of open reading frames, that encode polypeptides. Putative polypeptides may be identified based on polynucleotide sequences and further characterized. The sequencing data relating to polynucleotides thus represents valuable and useful information.

Polynucleotides and polypeptides may be analyzed for varying degrees of novelty by comparing identified sequences to sequences published in various public domain databases, such as EMBL. Newly identified polynucleotides and corresponding putative polypeptides may also be compared to polynucleotides and polypeptides contained in public domain information to ascertain homology to known polynucleotides and polypeptides. In this way, the degree of similarity, identity or homology of polynucleotides and polypeptides having an unknown function may be determined relative to polynucleotides and polypeptides having known functions.

Information relating to the sequences of isolated polynucleotides may be used in a variety of ways. Specified polynucleotides having a particular sequence may be isolated, or synthesized, for use in *in vivo* or *in vitro* experimentation as probes or primers. Alternatively, collections of sequences of isolated polynucleotides may be stored using magnetic or optical storage medium and analyzed or manipulated using computer hardware and software, as well as other types of tools.

Summary of the Invention

The present invention provides isolated polynucleotides comprising a sequence selected from the group consisting of: (a) sequences identified in the attached Sequence Listing as SEQ ID NOS: 1-80; (b) variants of those sequences; (c) extended sequences comprising the sequences set out in SEQ ID NOS: 1-80, and their variants; and (d) sequences comprising at least a specified number of contiguous residues of a sequence of SEQ ID NOS: 1-80 (*x*-mers). Oligonucleotide probes and primers corresponding to the sequences set out in SEQ ID NOS: 1-80, and their variants are also provided. All of these polynucleotides and oligonucleotide probes and primers are collectively referred to herein, as "polynucleotides of the present invention."

The polynucleotide sequences identified as SEQ ID NOS: 1-80 were derived from a microbial source, namely from fragmented genomic DNA of *Lactobacillus rhamnosus*, strain HN001, described in PCT International Publication No. WO 99/10476. *Lactobacillus rhamnosus* strain HN001 are heterofermentative bacteria that are Gram positive, non-motile, non-spore forming, catalase negative, facultative anaerobic rods exhibiting an optimal growth temperature of 37±1°C and an optimum pH of 6.0 – 6.5. Experimental studies demonstrated that dietary supplementation with *Lactobacillus rhamnosus* strain HN001 induced a sustained enhancement in several aspects of both natural and acquired immunity. A biologically pure culture of *Lactobacillus rhamnosus* strain HN001 was deposited at the Australian Government Analytical Laboratories (AGAL), The New South Wales Regional Laboratory, 1 Suakin Street, Pymble, NSW 2073, Australia, as Deposit No. NM97/09514, dated 18 August 1997.

Certain of the polynucleotide sequences disclosed herein are "partial" sequences in that they do not represent a full-length gene encoding a full-length polypeptide. Such partial sequences may be extended by analyzing and sequencing various DNA libraries using primers and/or probes and well-known hybridization and/or PCR techniques. The partial sequences disclosed herein may thus be extended until an open reading frame encoding a polypeptide, a full-length polynucleotide and/or gene capable of expressing a polypeptide, or another useful portion of the genome is identified. Such extended sequences, including full-length polynucleotides and genes, are described as "corresponding to" a sequence identified as one of the sequences of SEQ ID NOS: 1-80 or a variant thereof, or a portion of one of the sequences of SEQ ID NOS: 1-80 or a variant thereof, when the extended polynucleotide comprises an identified sequence or its variant, or an identified contiguous portion (*x*-mer) of one of the sequences of SEQ ID NOS: 1-80 or a variant thereof.

The polynucleotides identified as SEQ ID NOS: 1-80 were isolated from *Lactobacillus rhamnosus* genomic DNA clones and represent sequences that are present in the cells from which the DNA was prepared. The sequence information may be used to identify and isolate, or synthesize, DNA molecules such as promoters, DNA-binding elements, open reading frames or full-length genes, that then can be used as expressible or otherwise functional DNA in transgenic organisms. Similarly, RNA sequences, reverse sequences, complementary sequences, antisense

sequences and the like, corresponding to the polynucleotides of the present invention, may be routinely ascertained and obtained using the polynucleotides identified as SEQ ID NOS: 1-80.

The present invention further provides isolated polypeptides encoded, or partially encoded, by the polynucleotides disclosed herein. In certain specific embodiments, the polypeptides of the present invention comprise a sequence selected from the group consisting of sequences identified as SEQ ID NO: 81-183, and variants thereof. Polypeptides encoded by the polynucleotides of the present invention may be expressed and used in various assays to determine their biological activity. Such polypeptides may be used to raise antibodies, to isolate corresponding interacting proteins or other compounds, and to quantitatively determine levels of interacting proteins or other compounds.

Genetic constructs comprising the inventive polynucleotides are also provided, together with transgenic host cells comprising such constructs and transgenic organisms, such as microbes, comprising such cells.

The present invention also contemplates methods for modulating the polynucleotide and/or polypeptide content and composition of an organism, such methods involving stably incorporating into the genome of the organism a genetic construct comprising a polynucleotide of the present invention. In one embodiment, the target organism is a microbe, preferably a microbe used in fermentation, more preferably a microbe of the genus *Lactobacillus*, and most preferably *Lactobacillus rhamnosus,* or other closely microbial related species used in the dairy industry. In a related aspect, methods for producing a microbe having an altered genotype and/or phenotype is provided, such methods comprising transforming a microbial cell with a genetic construct of the present invention to provide a transgenic cell, and cultivating the transgenic cell under conditions conducive to growth and multiplication. Organisms having an altered genotype or phenotype as a result of modulation of the level or content of a polynucleotide or polypeptide of the present invention compared to a wild-type organism, as well as components and progeny of such organisms, are contemplated by and encompassed within the present invention.

The isolated polynucleotides of the present invention may be usefully employed for the detection of lactic acid bacteria, preferably *L. rhamnosus*, in a sample material, using techniques well known in the art, such as polymerase chain reaction (PCR) and DNA hybridization, as detailed below.

The inventive polynucleotides and polypeptides may also be employed in methods for the selection and production of more effective probiotic bacteria; as "bioactive" (health-promoting) ingredients and health supplements for immune function enhancement; for reduction of blood lipids such as cholesterol; for production of bioactive material from genetically modified bacteria; as adjuvants; for wound healing; in vaccine development, particularly mucosal vaccines; as animal probiotics for improved animal health and productivity; in selection and production of genetically modified rumen microorganisms for improved animal nutrition and productivity, better flavor and improved milk composition; in methods for the selection and production of better natural food bacteria for improved flavor, faster flavor development, better fermentation characteristics, vitamin synthesis and improved textural characteristics; for the production of improved food bacteria through genetic modification; and for the identification of novel enzymes for the production of, for example, flavors or aroma concentrates.

The isolated polynucleotides of the present invention also have utility in genome mapping, in physical mapping, and in positional cloning of genes of more or less related microbes. Additionally, the polynucleotide sequences identified as SEQ ID NOS: 1-80, and their variants, may be used to design oligonucleotide probes and primers. Such oligonucleotide probes and primers have sequences that are substantially complementary to the polynucleotide of interest over a certain portion of the polynucleotide. Oligonucleotide probes designed using the polynucleotides of the present invention may be used to detect the presence and examine the expression patterns of genes in any organism having sufficiently similar DNA and RNA sequences in their cells, using techniques that are well known in the art, such as slot blot DNA hybridization techniques. Oligonucleotide primers designed using the polynucleotides of the present invention may be used for polymerase chain reaction (PCR) amplifications. Oligonucleotide probes and primers designed using the polynucleotides of the present invention may also be used in connection with various microarray technologies, including the microarray technology of Affymetrix (Santa Clara, CA).

The polynucleotides of the present invention may also be used to tag or identify an organism or derived material or product therefrom. Such tagging may be accomplished, for example, by stably introducing a non-disruptive non-functional heterologous polynucleotide

identifier into an organism, the polynucleotide comprising at least a portion of a polynucleotide of the present invention.

The polynucleotides of the present invention may also be used as promoters, gene regulators, origins of DNA replication, secretion signals, cell wall or membrane anchors for genetic tools (such as expression or integration vectors).

All references cited herein, including patent references and non-patent publications, are hereby incorporated by reference in their entireties.

Brief Description of the Drawings

Fig. 1 shows the nucleotide sequence for *L. rhamnosus* strain HN001 deoD purine nucleoside phosphorylase *AQ1* (SEQ ID NO: 78), showing ATG initiation and translation stop codons (boxed).

Fig. 2 shows the amino acid sequence for *L. rhamnosus* strain HN001 deoD purine nucleoside phosphorylase *AQ1* (SEQ ID NO: 181).

Fig. 3 shows the results of UV light exposure assay measuring relative viability in response to increasing doses of UV light for *AQ1*$^-$ HN001 strain (♦) and wild-type HN001 (■). Results indicate that the *AQ1*$^-$ HN001 mutant strain showed enhanced survival to exposure to UV light compared to wild-type HN001.

Fig. 4 shows the nucleotide sequence for *L. rhamnosus* strain HN001 relA GTP pyrophosphokinase gene *AM1* (SEQ ID NO: 79) showing ATG initiation and translation stop codons (boxed).

Fig. 5 shows the amino acid sequence of *L. rhamnosus* strain HN001 relA GTP pyrophosphokinase gene *AM1* (SEQ ID NO: 182).

Fig. 6 shows the results of UV light exposure assay measuring relative viability in response to increasing doses of UV light in *AM1*$^-$ HN001 strain (♦) and wild-type HN001 (■). Results indicate that the *AM1*$^-$ HN001 mutant strain showed enhanced survival to exposure to UV light compared to wild-type HN001.

Detailed Description

The polynucleotides disclosed herein were isolated by high throughput sequencing of DNA libraries from the lactic acid bacteria *Lactobacillus rhamnosus* as described in Example 1. Cell wall, cell surface and secreted components of lactic acid bacteria are known to mediate immune modulation, cell adhesion and antibacterial activities, resulting in many beneficial effects including: resistance to enteric pathogens; modulation of cancer, including colon cancer; anti-mutagenesis effects; reduction of small bowel bacterial overgrowth; modulation of auto-immune disorders; reduction in allergic disorders; modulation of urogenital infections, inflammatory bowel disorder, irritable bowel syndrome, *Helicobacter pylori* infection and hepatic encephalopathy; reduction of infection with pathogens; regulation of colonocyte proliferation and differentiation; reduction of mucosal permeability; and relief of constipation and diarrhea. These cell components include, but are not limited to, peptidoglycans, teichoic acids, lipoteichoic acids, polysaccharides, adhesion proteins, secreted proteins, surface layer or S-layer proteins, collagen binding proteins and other cell surface proteins, and antibacterial substances such as bacteriocins and organic acids produced by these bacteria. Polynucleotides involved in the synthesis of these proteins and in the synthesis, modification, regulation, transport, synthesis and/or accumulation of precursor molecules for these proteins can be used to modulate the immune effects, antibacterial, cell adhesion and competitive exclusion effects of the bacteria or of components that might be produced by these bacteria.

In order to function effectively as probiotic bacteria, *L. rhamnosus* HN001 must survive environmental stress conditions in the gastrointestinal tract, as well as commercial and industrial processes. Modification of particular polynucleotides or regulatory processes has been shown to be effective against a number of stresses including oxidative stress, pH, osmotic stress, dehydration, carbon starvation, phosphate starvation, nitrogen starvation, amino acid starvation, heat or cold shock and mutagenic stress. Polynucleotides involved in stress resistance often confer multistress resistance, i.e., when exposed to one stress, surviving cells are resistant to several non-related stresses. Bacterial genes and/or processes shown to be involved in multistress resistance include:

Intracellular phosphate pools - inorganic phosphate starvation leads to the induction of *pho* regulon genes, and is linked to the bacterial stringent response. Gene knockouts involving phosphate receptor genes appear to lead to multistress resistance.

Intracellular guanosine pools - purine biosynthesis and scavenger pathways involve the production of phosphate-guanosine compounds that act as signal molecules in the bacterial stringent response. Gene knockouts involving purine scavenger pathway genes appear to confer multistress resistance.

Osmoregulatory molecules - small choline-based molecules, such as glycine-betaine, and sugars, such as trehalose, are protective against osmotic shock and are rapidly imported and/or synthesized in response to increasing osmolarity.

Acid resistance - lactobacilli naturally acidify their environment through the excretion of lactic acid, mainly through the *cit* operon genes responsible for citrate uptake and utilization.

Stress response genes - a number of genes appear to be induced or repressed by heat shock, cold shock, and increasing salt through the action of specific promoters.

The isolated polynucleotides of the present invention, and genetic constructs comprising such polynucleotides, may be employed to produce bacteria having desired phenotypes, including increased resistance to stress and improved fermentation properties.

Many enzymes are known to influence dairy product flavor, functional and textural characteristics as well as general fermentation characteristics such as speed of growth, acid production and survival. These enzymes include those involved in the metabolism of lipids, polysaccharides, amino acids and carbohydrates as well as those involved in the lysis of the bacterial cells.

The isolated polynucleotides and polypeptides of the present invention have demonstrated similarity to polynucleotides and/or polypeptides of known function. The identity

and functions of the inventive polynucleotides based on such similarities are shown below in Table 1.

TABLE 1

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
1, 8, 53	81, 88, 145	Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma, or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: (survival of industrial processes, growth or storage in product formats, persistence in gut environment). Modified carbohydrate levels or functional properties. Altered metabolic properties. Modified lactose metabolism. Altered probiotic attributes. Improved fermentation properties or other industrially useful processes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health, lactose tolerance)	Homologue of lacG that encodes 6-phospho-beta-galactosidase (EC 3.2.1.85). LacG is part of the lactose metabolism, and hydrolyzes phospholactose, the product of a phosphor-enolpyruvate-dependent phosphotransferase system. It belongs to the glycosidase family 1 and contributes to flavor, including bitter flavor.
2	82	Production of bioactive or functional polypeptides. Removal of undesirable flavor characteristics. Production of desirable flavors.	Homologue of pepS, encoding an aminopeptidase (EC 3.4.11.-). PepS catalyzes the release of free amino acids from peptides. Aminopeptidases are exopeptidases

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health). Altered resistance to antibiotics.	and ubiquitous enzymes, frequently observed in animals, plants and microorganisms. They are involved in many different functions in the cell, such as protein maturation, protein turnover, hydrolysis of regulatory peptides, nitrogen nutrition, modulation of gene expression etc. and, consequently, are considered essential enzymes. The proteolytic system of lactic acid bacteria is essential for bacterial growth in milk but also for the development of the organoleptic properties of dairy products. PepS is involved both in bacterial growth by supplying amino acids, and in the development of flavor in dairy products, by hydrolyzing peptides (including bitter peptides) and liberating aromatic amino acids which are important precursors of aroma compounds (Fernandez-Espla and Rul, *Eur. J. Biochem.* 263:502-510, 1999).
3	83	Production of bioactive or functional polypeptides. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product	Homologue of PepC, encoding aminopeptidase C (EC 3.4.22.40). PepC is also known as bleomycin hydrolase, which inactivates bleomycin B2 (a cytotoxic glycometallopeptide) by hydrolysis of a carboxyamide bond of b-aminoalanine. It also has general aminopeptidase activity. PepC belongs to peptidase family C1; also known as the papain family of thiol proteases and is involved in flavor production. The proteolytic system of lactic acid bacteria is essential for bacterial growth in milk but also for the development of the organoleptic properties of dairy products.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		formats, persistence in gut environment. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health). Altered resistance to antibiotics.	
4	84	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	Homologue of hisC, coding for a L-histidinol phosphate aminotransferase (EC 2.6.1.9). The HisC aminotransferase is pyridoxal-5'-phosphate (PLP)-dependent and is involved in the synthesis of histidine in eubacteria. HisC also has tyrosine and phenylalanine aminotransferase activity. The biosynthesis of histidine is a central metabolic process in organisms ranging from bacteria to yeast and plants.
5	85	Production of desirable flavors. Modified flavor, aroma and/or	Homologue of mvaD coding for mevalonate pyrophosphate

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	decarboxylase (EC 4.1.1.33). MvaD is part of the mevalonate pathway for the biosynthesis of the central isoprenoid precursor, isopentenyl diphosphate by catalyzing the reaction of mevalonate 5-diphosphate with ATP to produce inorganic phosphate, ADP, CO_2 and isopentenyl diphosphate, the building block of sterol and isoprenoid biosynthesis.
6	86	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified adhesion to human or animal cells or cell lines. Organisms or materials with	Homologue of protein p60, encoded by the gene termed iap. The p60 protein is a major extracellular product secreted by all isolates of *L. monocytogenes*. This protein has peptidoglycan hydrolase activity but also influences the uptake of *L. monocytogenes* by non-phagocytic cells. Proteins related to p60 are also found in all other *Listeria* species. It has been shown that p60 protein is among the strongest antigens in listeriae for B- and T-cell responses. The protein p60 belongs to the *E.coli* nlpc / listeria p60 family.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		improved health properties (including immunoregulatory, anticancer, gut health) Altered resistance to antibiotics. Improved antimicrobial properties.	
7	87	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	Homologue of elongation factors Tu (EF-Tu) and 1 alpha (EF-1alpha) that are homologous proteins essential to translation in bacteria and eukaryotes, respectively. EF-Tu and EF-1alpha are GTPases that catalyze the binding of aminoacyl-tRNAs to the A-site of the ribosome. As they are among the slowest evolving proteins known, EFs are used to study cellular functions and to root the universal tree of life (Gaucher *et al. Proc. Natl. Acad. Sci. USA* 98:548-552, 2001), and are therefore an excellent genetic tool.
9	89	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment.	Homologue of ribonuclease HII (EC 3.1.26.4), an RNAse that specifically degrades the RNA moiety in RNA/DNA hybrids. Endogenous RNase H activity plays an essential role in biological effects mediated by antisense oligonucleotides, molecules considered as potential agents against infectious diseases and pathologies resulting from dysfunctional genes. The prokaryotic RNAse HII is the evolutionary counterpart of the major mammalian RNase H (Frank *et al., Proc. Natl. Acad. Sci. USA* 95:12872-12877, 1998) and is necessary for cell

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	survival.
10	90	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified lipid, glycolipid or free fatty acid levels or functional properties. Modified production of short chain fatty acids. Altered lipid metabolism. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	Lipase homologue. Lipases are enzymes that catalyze hydrolysis of fatty acid ester bonds in triacylglycerol (TAG) and releasing free fatty acids. The reaction is reversible and therefore the enzyme can catalyze esterification of glycerol to form mono, di and triglycerides. Free fatty acids are important in providing flavor-bearing compounds for dairy products such as cheese, and have a significant role in both flavor and texture. Used extensively in wide range of convenience foods. Short chain fatty acids are known to have a variety of health impacts.
11	91	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein	Homologue of hisD, encoding histidinol dehydrogenase (HDH, EC 1.1.1.23). HisD catalyzes the last two steps in the biosynthesis of L-histidine: sequential NAD-dependent oxidations of L-histidinol to L-histidinaldehyde and then to L-histidine. Because hisD is absent in mammals, it is a target for inhibition

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	as part of herbicide development (Barbosa *et al.*, *Proc. Natl. Acad. Sci. USA* 99:1859-1864, 2002).
11	92	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Regulation of amino acid metabolism. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	Homologue of hisZ, coding for an aminoacyl-tRNA synthetase. HisZ is an essential component of the first enzyme in histidine biosynthesis with ATP phosphoribosyltransferase (HisG, EC 2.4.2.17) but lacks aminoacylation activity. HisZ is also called an ATP phosphoribosyltransferase regulatory subunit. HisZ is required for histidine prototrophy and directly involved in the transferase function. Both HisG and HisZ are required for catalyzing the ATP phosphoribosyltransferase reaction. Aminoacyl-tRNA synthetases have an essential catalytic role in protein biosynthesis, but also participate in numerous other functions, including regulation of gene expression and amino acid biosynthesis via transamidation pathways (Sissler *et al.*, *Proc. Natl. Acad. Sci. USA* 96:8985-8990, 1999). Because HisD is absent in mammals, it is a target for inhibition as part of herbicide development (Barbosa *et*

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
			al., Proc. Natl. Acad. Sci. USA 99:1859-1864, 2002).
12	93	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties.	Homologue of proA, coding for a glutamate-5-semialdehyde dehydrogenase (EC 1.2.1.41). ProA is also known as gamma-glutamylphosphate reductase, and catalyzes the second step of proline biosynthesis, the NADPH-dependent reduction of L-gamma-glutamyl 5-phosphate into L-glutamate 5-semialdehyde and phosphate. Intracellular accumulation of the amino acid proline has been linked to salt tolerance and virulence potential of a number of bacteria. Proline biosynthesis plays an important role in survival in osmolyte-depleted environments of elevated osmolarity. The survival of the food-borne pathogen *L. monocytogenes* in hypersaline environments is attributed mainly to the accumulation of organic compounds termed osmolytes. Osmolytes, often referred to as compatible solutes owing to their compatibility with cellular metabolism at high internal concentrations, can be either transported into the cell or synthesized *de novo* and act by counterbalancing the external osmotic strength, thus preventing water loss and plasmolysis. As well as its role as an osmoprotectant, proline may function as a virulence factor for certain pathogenic bacteria (Sleator *et al., Appl. Environ. Microbiol.* 67:2571-2577, 2001).
12	94	Altered amino acid metabolism.	Homologue of proB, coding for

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties.	gamma-glutamyl kinase (EC 2.7.2.11) also known as glutamate 5-kinase 1. ProB catalyzes the first step of proline biosynthesis, the transfer of a phosphate group to glutamate to form glutamate 5-phosphate which rapidly cyclizes to 5-oxoproline. Intracellular accumulation of the amino acid proline has been linked to the salt tolerance and virulence potential of a number of bacteria. Proline biosynthesis plays an important role in survival in osmolyte-depleted environments of elevated osmolarity. The survival of the food-borne pathogen *L. monocytogenes* in hypersaline environments is attributed mainly to the accumulation of organic compounds termed osmolytes. Osmolytes, often referred to as compatible solutes owing to their compatibility with cellular metabolism at high internal concentrations, can be either transported into the cell or synthesized *de novo* and act by counterbalancing the external osmotic strength, thus preventing water loss and plasmolysis. As well as its role as an osmoprotectant, proline may function as a virulence factor for certain pathogenic bacteria (Sleator *et al., Appl. Environ. Microbiol.* 67:2571-2577, 2001).
13	95	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors.	Homologue of vanHE or LDHD, encoding a D-lactate dehydrogenase (D-LDH, EC 1.1.1.28). D-LDH reduces pyruvate to D-lactate and is involved in bacterial cell wall structure and function. VanH plays an

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties.	essential role in bacterial resistance to the antibiotic vancomycin.
14	96	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties.	Homologue of metA that encodes homoserine O-transsuccinylase (EC 2.3.1.46). MetA catalyzes the first unique step in bacterial and plant methionine biosynthesis involving the activation of the gamma-hydroxyl of homoserine. The activity of this enzyme is closely regulated *in vivo* and therefore represents a critical control point for cell growth and viability.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer gut health). Modified antibiotic resistance.	
15	97	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer gut health). Modified antibiotic resistance. Improved antimicrobial properties.	Homologue of plnG, encoding an ATP binding cassette (ABC) transporter for the antimicrobial compound (bacteriocin) plantaricin A. PlnG displays strong similarities to the proposed transport proteins of several other bacteriocins and to proteins implicated in the signal-sequence-independent export of *Escherichia coli* hemolysin, PlnH is its accessory protein (Huhne *et al., Microbiol.* 142:1437-1448, 1996).
16	98	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes,	Homologue of hemN that encodes an oxygen-independent coproporphyrinogen III dehydrogenase (EC 1.3.3.3). HemN catalyzes the oxidative decarboxylation of coproporphyrinogen III to yield protoporphyrinogen IX and requires NADP+, ATP, Mg2+, and L-methionine. In association with specific apoproteins, it serves a wide range of important functions

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		growth or storage in product formats, persistence in gut environment. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes. Altered antimicrobial properties. Modified health properties (including immunoregulatory, anticancer gut health).	including electron transport (e.g., cytochromes), binding and transport of O_2 (e.g., hemoglobin), and oxidative catalysis (e.g., peroxidases) (Fischer *et al., J. Bacteriol.* 183:1300-1311, 2001).
17	99	Removal of undesirable flavor characteristics. Modified flavor, aroma, texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: (survival of industrial processes, growth or storage in product formats, persistence in gut environment). Modified carbohydrate levels or functional properties. Altered metabolic properties. Modified lactose metabolism. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health, lactose tolerance).	Homologue of lacD, encoding tagatose-1,6-bisphosphate aldolase (EC 4.1.2.40). LacD is responsible for the aldol cleavage of tagatose-1,6-bisphosphate to form glycerone-P and glyceraldehyde 3-phosphate in the tagatose 6-phosphate pathway of lactose catabolism in bacteria. The enzyme activity is stimulated by certain divalent cations.
18	100	Altered amino acid metabolism. Removal of undesirable flavor characteristics.	Homologue of asnH, encoding asparagine synthetase [glutamine-hydrolyzing] 2 (EC 6.3.5.4). AsnH

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Altered cell wall or cell surface characteristics, structures or functions. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Improved fermentation properties or other industrially useful processes.	transamidates asparagine, glutamate, AMP and diphosphate from aspartate, glutamine and ATP as part of the asparagine biosynthesis pathway. AsnH gene is also involved in cell surface organization.
19	101	Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product	Homologue of butB, encoding 2,3-butanediol dehydrogenase (EC 1.1.1.4). ButB catalyzes the NAD+-dependent oxidation of 2,3-butanediol acetoin, as well as the corresponding reverse reactions. It can also reduce diacetyl to acetoin. Diacetyl is an important flavor compound in dairy products.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	
20	102	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	Homologue of a peptidoglycan hydrolase (N-acetylmuramoyl-L-alanine amidase). N-acetylmuramoyl-L-alanine amidase is an autolysin involved in degrading the cell wall during cell growth or programmed cell death and is involved in cell growth and important for releasing enzymes important for flavor.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
21, 76	103, 175	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health, apoptosis). Modified antibiotic resistance. Improved antimicrobial properties. Improved fermentation properties or other industrially useful processes.	Homologue of galC, encoding galactosylceramidase (EC 3.2.1.46). GalC hydrolyzes galactose ester bonds of galactosylceramide, galactosylsphingosine, monogalactosyldiglyceride and lactosylceramide. It is involved in the catabolism of galactosylceramide, a major lipid in myelin, kidney and epithelial cells of small intestine and colon. While bacteria may use galC to release sugars for metabolism, the by-products, including ceramide, asct as signalling molecules in eukaryotic cells and can lead to apoptosis or differentiation. Therefore, glaC plays a role in probiotic effects and survival in the gut environment.
22	104	Production of bioactive or functional polypeptides. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein	Homologue of pepN, encoding membrane alanyl aminopeptidase (EC 3.4.11.2), also called lysyl aminopeptidase and aminopeptidase N. PepN releases the N-terminal amino acid, Xaa-|-Xbb- from a peptide, amide or arylamide. Aminopeptidases are involved in many different functions in the cell, such as protein maturation, protein

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health). Altered resistance to antibiotics.	turnover, hydrolysis of regulatory peptides, nitrogen nutrition, modulation of gene expression etc. and, consequently, are considered essential enzymes. The proteolytic system of lactic acid bacteria is essential for bacterial growth in milk but also for the development of the organoleptic properties of dairy products. PepN is involved both in bacterial growth by supplying amino acids, and in the development of flavor in dairy products, by hydrolyzing peptides (including bitter peptides) and liberating aromatic amino acids which are important precursors of aroma compounds (Fernandez-Espla and Rul, *Eur. J. Biochem.* 263:502-510, 1999).
23	105	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory	Homologue of mvaB, encoding 3-hydroxy-3-methylglutaryl coenzyme A synthase. MvaB catalyzes the condensation of acetyl-CoA with acetoacetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA and CoA. MvaB is involved in mevalonic acid metabolism as well as in biosynthesis of cholesterol and ubiquinone progenitors. Terpenoids or isoprenoids constitute a vast family of organic compounds that includes sterols and carotenoids that have flavor, color, texture and other sensory impacts on food products. The terpenoids in many organisms share early steps in their biosynthesis, including the synthesis of 3-hydroxy-3-methylglutaryl-coenzyme A and its conversion to mevalonate.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		anticancer, gut health).	
24	106	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	Homologue of hexB, one of two proteins involved in DNA mismatch repair. The hex mismatch repair system of *Streptococcus pneumoniae* acts both during transformation (a recombination process that directly produces heteroduplex DNA) to correct donor strands and after DNA replication to remove misincorporated nucleotides. The hexB is one of at least two proteins required for mismatch repair. HexB is homologous to the mutL protein, which is required for methyl-directed mismatch repair in *Salmonella typhimurium* and *E. coli*, and to the PMS1 gene product, which is likely to be involved in a mismatch correction system in *Saccharomyces cerevisiae* (Prudhomme *et al., J. Bacteriol.* 171:5332-5338, 1989).
25	107	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment.	Homologue of araT, encoding an aromatic amino acid aminotransferase (EC 2.6.1.57). Aminotransferases have been widely applied in the large-scale biosynthesis of amino acids, which are in increasing demand by the pharmaceutical industry. AraT plays a major role in the conversion of aromatic amino acids to aroma compounds. AraT also has a major physiological role in the biosynthesis of phenylalanine and tyrosine. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Indeed, degradation products from aromatic, branched-chain, and sulfurous amino acids

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	have been identified in various cheeses and highly contribute to their flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
26	108	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	Homologue of tagE, encoding poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52) also called uridine diphosphate-glucose poly-(glycerol phosphate) alpha-glucosyl transferase. TagE is involved in techoic acid synthesis. Techoic acid is one component of the thick peptidoglycan layers in the cell wall of Gram-positive bacteria and is susceptible to the enzyme lysozyme and to penicillin.
27	109	Altered amino acid metabolism. Removal of undesirable flavor	Homologue to hisB, encoding a histidine biosynthesis bifunctional

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	protein –includes: imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19) and histidinol-phosphatase (EC 3.1.3.15). HisB rearranges the imidazole glycerol phosphate by a redox-neutral dehydrative reaction to imidazole acetol phosphate and catalyzes the dephosphorylation of hisidinol phosphate to histidinol, the direct precursor of histidine. Because hisB is absent from mammals, it has become a target for inhibition as part of herbicide development (Barbosa *et al., Proc. Natl. Acad. Sci. USA* 99:1859-1864, 2002). Amino acid degradation products in various cheeses have been shown to greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
28	110	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties.	Homologue of cysK, encoding cysteine synthase (EC 4.2.99.8), also known as O-acetyl-L-serine acetate-lyase (EC 4.2.99.8). CysK catalyzes the formation of L-cysteine, the last step of L-cysteine biosynthesis, from O-acetyl-L-serine and hydrogen sulfide. Cysteine synthase is involved in the assimilatory sulfate reduction pathway and in the selenium incorporation into proteins, which occurs mainly as selenocysteine, in bacteria. Sulphur-containing amino acid metabolism is important for development of aroma and flavor compounds.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	
29	111	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties.	Homologue of enn protein. Enn has unique Ig-binding characteristics as it reacts preferentially with human IgG3, the tlpC gene. Enn is a membrane protein with similarity to methyl-accepting chemotaxis proteins and the streptococcal M proteins homologous with immunoglobulin-binding factors. The M proteins have been studied because of their antiphagocytic function.
30	112	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors	Homologue of hisE, encoding a histidine biosynthesis protein that plays a role in histidine biosynthesis. Because hisE is absent in mammals, it is a target for inhibition as part of herbicide development (Barbosa *et al., Proc. Natl. Acad. Sci. USA*

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	99:1859-1864, 2002). Amino acid degradation products in various cheeses have been shown to greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
30	113	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	Homologue of hisI, encoding a histidine biosynthesis protein that plays a role in histidine biosynthesis. Because hisI is absent in mammals, it is a target for inhibition as part of herbicide development (Barbosa *et al., Proc. Natl. Acad. Sci. USA* 99:1859-1864, 2002). Amino acid degradation products in various cheeses have been shown to greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
31	114	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified lipid, glycolipid or free fatty acid levels or functional properties. Modified production of short chain fatty acids. Altered lipid metabolism. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	Homologue of estA, encoding a serine-dependent arylesterase (EC 3.1.1.2). EstA hydrolyzes a variety of ester compounds and prefers those with substituted phenyl alcohol or short-chain fatty acid groups. Arylsesterases are responsible for the production of important flavor compounds and intermediates.
32	115	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations.	Homologue of glnA that encodes a glutamine synthetase (EC 6.3.1.2), also called glutamate-ammonia ligase. GlnA catalyzes the first step in the conversion of inorganic nitrogen (ammonium) into its organic form glutamine (Gln). Bacterial glutamine synthetase export is associated with pathogenicity and with the formation of a poly-L-glutamate/glutamine cell wall structure. Glutamine synthetase is an enzyme that plays a central role in the nitrogen metabolism. The

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Altered cell wall or cell surface characteristics, structures or functions.	enzyme and its products have roles in flavor and growth.
32	116	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties.	Homologue of Lipopolysaccharide synthesis protein yohJ. YohJ is involved in techoic acid synthesis and important for cell wall functions including adhesion, immune cell interaction and product texture.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
33	117	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	Homologue of sorA. SorA encodes the first protein of the phosphoenolpyruvate-dependent L-sorbose-specific phosphotransferase system (PTS). The ketose L-sorbose is transported and phosphorylated through PTS. The enzyme is useful in carbohydrate-specific regulation of gene expression and flavor development.
33	118	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or	Homologue of sorF, encoding D-sorbitol-6-phosphate dehydrogenase, an enzyme of the L-sorbose metabolism. The ketose L-sorbose is transported and phosphorylated through the phosphoenolpyruvate-dependent L-sorbose-specific phosphotransferase system (PTS). Useful for carbohydrate-specific regulation of gene expression (Yebra *et al.*, *J. Bacteriol.* 182:155-163, 2000).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		functional properties. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	
34	119	Production of bioactive or functional polypeptides. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	Homologue of pepA, encoding a glutamyl aminopeptidase, which belongs to peptidase family M42 and is required for optimal growth of *Lactococcus lactis* MG1363 in milk. PepA has DNA-binding activity that functions in transcription control and plasmid dimer resolution. Aminopeptidases are involved in many different functions in the cell, such as protein maturation, protein turnover, hydrolysis of regulatory peptides, nitrogen nutrition, modulation of gene expression etc. and, consequently, are considered essential enzymes. The proteolytic system of lactic acid bacteria is essential for bacterial growth in milk but also for the development of the organoleptic properties of dairy products. PepA is involved both in bacterial growth by supplying amino acids, and in the development of flavor in dairy products, by hydrolyzing peptides (including bitter peptides) and liberating aromatic amino acids which are important precursors of aroma compounds (Fernandez-Espla and Rul, *Eur. J. Biochem.* 263:502-510, 1999).
35	120	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors.	Homologue of hom, encoding a homoserine dehydrogenase (EC 1.1.1.3), an enzyme of the threonine biosynthesis pathway. Threonine is

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health).	derived from aspartic acid. Conversion of aspartate to homoserine proceeds with ATP activation of the B-carboxyl group as a mixed phosphoric anhydride followed by two sequential NADPH-dependent reductions to homoserine. Phosphorylation of homoserine provides the substrate phosphohomoserine which suffers a stereospecific 1,2-transposition reaction to give threonine. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
35	121	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes.	Homologue of flotillin. Flotillins behave as resident integral membrane protein components of caveolae which are plasmalemmal microdomains and are involved in vesicular trafficking and signal transduction (Huang *et al., Mol. Microbiol.* 31:361-371, 1999). Flotillins (also known as epidermal surface antigens (ESAs)) belong to the family of caveolae-associated integral membrane proteins and may act as a scaffolding protein within caveolar membranes.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties.	
36	122	Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Improved fermentation properties or other industrially useful processes.	Homologue of areB, encoding benzyl alcohol dehydrogenase (EC 1.1.1.90). AreB catalyzes the oxidation of an aromatic alcohol to an aromatic aldehyde. This enzyme enables bacteria to grow on a range of esters of aromatic alcohols and plays a role in flavor development.
37	123	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein	Homologue of codB, encoding a cytosine permease. CodB mediates uptake of exogenously supplied cytosine. It belongs to the ABC transporter family. The cytosine permease is an integral cytoplasmic membrane protein possessing several transmembrane-spanning domains. The enzymatic degradation of amino

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered response to stress conditions. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties. Improved fermentation properties or other industrially useful processes.	acids in cheese plays a major role in cheese flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
38	124	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic	Homologue of hsp18, encoding a low-molecular-weight protein belonging into a family of small heat shock proteins. Hsp18 is induced not only by heat shock but also at the onset of solventogenesis. Small heat shock proteins (sHsps) are a diverse group of heat-induced proteins that are conserved in prokaryotes and eukaryotes and are especially abundant in plants. Recent *in vitro* data indicate that sHsps act as molecular chaperones to prevent thermal aggregation of proteins by binding non-native intermediates, which can then be refolded in an ATP-dependent fashion by other chaperones (Lee and Vierling, *Plant. Physiol.* 122:189-198, 2000).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		pathways. Altered probiotic attributes.	
39	125	Altered cell wall or cell surface characteristics, structures or functions. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties.	Homologue to mccF, encoding a inner membrane associated protein of bacteria. MccF determines resistance to exogenous microcin. Possible action by preventing the reentering of the cell by exported translation inhibitor microcin C7 (Gonzalez-Pastor *et al., J. Bacteriol.* 177:7131-7140, 1995).
40	126	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product	Homologue of sorE, encoding an L-sorbose-1-phosphate reductase. SorF is, together with D-glucitol-6-phosphate dehydrogenase, involved in the conversion of L-sorbose-1-phosphate to D-fructose-6-phosphate. SorE is involved in flavor development and carbohydrate metabolism.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	
41	127	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	Homologue of the iolB gene, encoding a cis-acting catabolite-responsive element (cre) protein involved in the iol operon of the myo-inositol catabolism pathway. Myo-inositol is abundant in nature, especially in soil. Various microorganisms are able to grow on myo-inositol as the sole carbon source. The expression of the iol operon is under glucose repression (Miwa and Fujita, *J. Bacteriol.* 183:5877-5884, 2001).
41	128	Production of desirable flavors. Modified flavor, aroma and/or	Homologue of the iolC gene, encoding 2-dehydro-3-

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	deoxygluconokinase. IolC phosphorylates the 2-deoxy-5-keto-D-gluconic acid to 2-deoxy-5-keto-D-gluconic acid 6-phosphate and is part of the iol operon of the myo-inositol catabolism pathway (Yoshida *et al.*, *J. Bacteriol.* 179:4591-4598, 1997). Myo-inositol is abundant in nature, especially in soil. Various microorganisms are able to grow on myo-inositol as the sole carbon source. The expression of the iol operon is under glucose repression (Miwa and Fujita, *J. Bacteriol.* 183:5877-5884, 2001).
41	129	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment.	Homologue of the iolE gene, encoding a protein involved in the iol operon of the myo-inositol catabolism pathway. Myo-inositol is abundant in nature, especially in soil. Various microorganisms are able to grow on myo-inositol as the sole carbon source. The expression of the iol operon is under glucose repression (Miwa and Fujita, *J. Bacteriol.* 183:5877-5884, 2001).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	
41	130	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	Homologue of the iolG gene, encoding inositol dehydrogenase (EC 1.1.1.18). IolG catalyzes the first reaction of the inositol catabolism, the dehydrogenation of myo-inositol into 2-keto-myo-inositol (2-inosose) (Yoshida *et al., J. Bacteriol.* 179:4591-4598, 1997). IolG is part of the iol operon of the myo-inositol catabolism pathway. Myo-inositol is abundant in nature, especially in soil. Various microorganisms are able to grow on myo-inositol as the sole carbon source. The expression of the iol operon is under glucose repression (Miwa and Fujita, *J. Bacteriol.* 183:5877-5884, 2001).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
41	131	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	Homologue of the iolJ gene, encoding fructose-bisphosphate aldolase (EC 4.1.2.13) or tagatose-bisphosphate aldolase (4.1.2.40). IolJ cleaves 2-deoxy-5-keto-D-gluconic acid 6-phosphate to yield dihydroxyacetone phosphate and malonic semialdehyde and is part of the iol operon of the myo-inositol catabolism pathway (Yoshida *et al.*, *J. Bacteriol.* 179:4591-4598, 1997). Myo-inositol is abundant in nature, especially in soil. Various microorganisms are able to grow on myo-inositol as the sole carbon source. The expression of the iol operon is under glucose repression (Miwa and Fujita, *J. Bacteriol.* 183:5877-5884, 2001).
41	132	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other	Homologue of mmsA or iolA, encoding methylmalonate-semialdehyde dehydrogenase. IolA converts malonic semialdehyde into acetyl CoA and CO_2, the final step of inositol degradation (Yoshida *et al.*, *J. Bacteriol.* 179:4591-4598, 1997). Myo-Inositol is abundant in nature, especially in soil. Various microorganisms are able to grow on myo-inositol as the sole carbon source. The expression of the iol

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	operon is under glucose repression (Miwa and Fujita, *J. Bacteriol.* 183:5877-5884, 2001). The enzyme is also required for growth on valine and isoleucine as it is an acylating enzyme that converts both propanal and 2-Methyl-3-oxopropanoate to Propanoyl-CoA. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
42	133	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory,	Homologue of hisF, encoding imidazole glycerol phosphate synthase subunit hisF (EC 4.1.3.-) also called IGP synthase cyclase subunit. HisF links histidine and de novo purine biosynthesis and is a member of the glutamine amidotransferase family. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		anticancer, gut health). Modified antibiotic resistance.	
43	134	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes. Improved fermentation properties or other industrially useful processes. Modified health properties (including immunoregulatory, anticancer, gut health).	Homologue of yqcM, encoding arsenate reductase (EC 1.97.1.5). YqcM reduces the arsenate ion (H_2AsO) to arsenite ion (AsO). Arsenate is an abundant oxyanion that, because of its ability to mimic the phosphate group, is toxic to cells. Arsenate reductase participates to achieve arsenate resistance in both prokaryotes and yeast by reducing arsenate to arsenite; the arsenite is then exported by a specific transporter. Arsenite reductase is coupled to the glutathione and glutaredoxin system for its enzyme activity (Bennett *et al., Proc. Natl. Acad. Sci. USA* 98:13577-13582, 2001).
44	135	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of	Homologue of cna, encoding a collagen adhesin. Cna mediates attachment of bacterial cells to collagen-containing substrata and is attached to the cell wall peptidoglycan by an amide bond.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties.	
45, 65	136, 159	Production of bioactive or functional polypeptides. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory)	Homologue of pepQ, encoding a Xaa-Pro dipeptidase (EC 3.4.13.9). PepQ hydrolyzes Xaa-Pro dipeptides (but not Pro-Pro) and also acts on aminoacyl-hydroxyproline analogs This peptidase belongs to peptidase family M24A (methionyl aminopeptidase family). It has a potential use in the dairy industry as a cheese-ripening agent since proline release from proline-containing peptides in cheese reduces bitterness. The proteolytic system of lactic acid bacteria is essential for bacterial growth in milk but also for the development of the organoleptic properties of dairy products. PepQ is involved both in bacterial growth by supplying amino acids, and in the development of flavor in dairy products, by hydrolyzing peptides (including bitter peptides) and liberating aromatic amino acids which are important precursors of aroma compounds (Fernandez-Espla and Rul, *Eur. J. Biochem.* 263:502-

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
			510, 1999).
46	137	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	Homologue of argG, encoding a argininosuccinate synthase (EC 6.3.4.5). ArgG catalyzes the penultimate step of the arginine biosynthesis. It belongs to the argininosuccinate synthase family. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
46	138	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes,	Homologue of argH, encoding argininosuccinate lyase (EC 4.3.2.1). ArgH catalyzes the last step in the arginine biosynthesis. Argininosuccinate lyase also participates in the urea cycle, the major pathway for the detoxification of ammonia, where it catalyzes the reversible breakdown of argininosuccinic acid into arginine and fumarate. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Amino acid degradation products greatly

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
47	139	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	Homologue of purL, encoding a phosphoribosylformylglycinamidine (FGAM) synthetase (EC 6.3.5.3). PurL catalyzes the fourth step in the biosynthesis of purines. It is involved in multistress resistance. Purines play essential roles in many cellular functions, including DNA replication, transcription, intra- and extra-cellular signaling, energy metabolism, and as coenzymes for many biochemical reactions.
48	140	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors	Homologue of hisH, encoding a imidazole glycerol phosphate synthase subunit that is also known as IGP synthase glutamine amidotransferase subunit. HisH catalyzes the fifth step of the histidine biosynthesis. The hisH subunit

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	provides the glutamine amidotransferase activity that produces the ammonia necessary to hisF for the synthesis of IGP and AICAR. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
49	141	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	Homologue of ndK, encoding nucleoside-diphosphate kinase (EC 2.7.4.6), which converts (deoxy)ribonucleoside diphosphates into their corresponding triphosphates. NdK is an ubiquitous and nonspecific enzyme but is an important cellular enzyme that monitors and maintains nucleotide pools and has been implicated in a number of regulatory processes, including signal transduction, development and cell surface polysaccharide synthesis.
50	142	Altered cell wall or cell surface characteristics, structures or	Homologue of PrtB, a PII-type proteinase precursor (Lactocepin)

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	also called cell wall-associated serine proteinase (EC 3.4.21.96). PrtB breaks down milk proteins during the growth of the bacteria on milk and that provides the peptides essential for cell growth. It has endopeptidase activity with very broad specificity. It is best known for its action on caseins, although it has been shown to hydrolyze hemoglobin and oxidized insulin b-chain. Lactocepin is a type I membrane protein, located in the cell wall and belongs to peptidase family S8; also known as the Subtilase Family. Lactocepin is responsible for the hydrolysis of casein in milk and specificity differences between lactocepins from different starter strains may be partly responsible for imparting different flavor qualities to cheese (Broadbent *et al., Appl. Environ. Microbiol.* 68:1778-1785, 2002).
51	143	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics:	Homologue of aspB, encoding an aspartate aminotransferase (EC: 2.6.1.1), also called aspartate transaminase. AspB catalyzes the amino group transfer between amino acids and 2-oxo acids and that plays a central role in amino acid metabolism in organisms. The transferase is important for the metabolism of amino acids and Krebs cycle related organic acids. It plays a role in the production of important flavor determinants. The enzymatic degradation of amino acids in cheese

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	plays a major role in cheese flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
52	144	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	Homologue of iolF, encoding an inositol transporter (TC#: 2.A.1.1.27). IolF transports myo-inositol into the bacterial cell. IolF is part of the iol operon of the myo-inositol catabolism pathway. Myo-inositol is abundant in nature, especially in soil. Various microorganisms are able to grow on myo-inositol as the sole carbon source. The expression of the iol operon is under glucose repression (Miwa and Fujita, *J. Bacteriol.* 183:5877-5884, 2001).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
54	146	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties. Regulation of polysaccharide production, adhesion, immune modulation.	Homologue of mga4, a positive regulatory protein that acts as a component of a signal transducing system. Positive regulatory proteins or activator proteins bind in their active state to DNA in the promoter region and help RNA polymerase to bind and transcribe that gene. Mga4 initiates transcription of surface-associated/virulence factors.
55	147	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein	Homologue of BH3554, encoding a carboxylesterase (3.1.1.1). BH3554 hydrolyzes carboxylic ester bonds with relatively broad substrate specificity. It is involved in amino acid metabolism and flavor. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Amino acid

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
56	148	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health).	Homologue of Amd1, encoding an aminoacylase (EC 3.5.1.14). Amd1 deacetylates acylated amino acids. It plays a role in the production of important flavor determinants. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	
57	149	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	Homologue of tmpA, encoding a putative transmembrane protein. TmpA plays a role in adhesion and is part of an operon containing the mapA gene.
58	150	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations.	Homologue of npr, encoding a NADH peroxidase (EC 1.11.1.1). Npr utilizes hydrogen peroxide to create water and nicotinamide adenine dinucleotide (NADH) from its oxidized form (NAD). Metabolism of co-factors such as NADH can greatly

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes. Improved fermentation properties or other industrially useful processes.	influence the speed and type of metabolic pathway utilized under different redox conditions, and can therefore influence flavor and/or functionality.
59, 60	151, 152	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	Homologue of nifS. NifS is involved in cysteine metabolism and development of flavor compounds. The enzymatic degradation of amino acids in cheese plays a major role in cheese flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Improved fermentation properties or other industrially useful processes.	
61	153	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified lipid, glycolipid or free fatty acid levels or functional properties. Modified production of short chain fatty acids. Altered lipid metabolism. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health)	Homologue of fabA, encoding a 3-hydroxydecanoyl-ACP dehydratase. FabA introduces cis unsaturation into fatty acids during saturated fatty acid biosynthesis. The dehydratase belongs to the thioester dehydratase family. Free fatty acids are important in providing flavor-bearing compounds for dairy products such as cheese, and have a significant role in both flavor and texture. Used extensively in wide range of convenience foods. Short chain fatty acids are known to have a variety of health impacts.
62	154	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA	Homologue of aggH, encoding an autoaggregation mediating protein. AggH contains a region of similarity to ATP-dependent DEAD-box helicase. The protein is involved in genetic exchange, pathogen exclusion and persistence in the gut environment by promoting aggregation between bacteria.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties. Improved fermentation properties or other industrially useful processes.	
63	155	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered cell wall or cell surface characteristics, structures or	Homologue of the iolH gene, encoding a protein involved in the iol operon of the myo-inositol catabolism pathway. Myo-inositol is abundant in nature, especially in soil. Various microorganisms are able to grow on myo-inositol as the sole carbon source. The expression of the iol operon is under glucose repression (Miwa and Fujita, *J. Bacteriol.* 183:5877-5884, 2001)

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		functions. Modified adhesion to human or animal cells or cell lines. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	
63	156	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified carbohydrate levels or functional properties. Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	Homologue of the iolL gene, encoding a protein involved in the iol operon of the myo-inositol catabolism pathway. Myo--nositol is abundant in nature, especially in soil. Various microorganisms are able to grow on myo-inositol as the sole carbon source. The expression of the iol operon is under glucose repression (Miwa and Fujita, *J. Bacteriol.* 183:5877-5884, 2001)
64	157	Removal of undesirable flavor characteristics. Modified flavor, aroma, texture attributes.	Homologue of citX, encoding apo-citrate lyase phosphoribosyl-dephospho-CoA transferase (Apo-ACP nucleodityltransferase; EC

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: (survival of industrial processes, growth or storage in product formats, persistence in gut environment). Modified carbohydrate levels or functional properties. Altered metabolic properties. Modified citrate metabolism. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health). Improved fermentation properties or other industrially useful processes.	2.8.3.10). The transferase belongs to the citX family (Schneider *et al.*, *Biochem.* 39:9438-9450, 2000). Carbohydrate metabolism impacts on flavor, functionality and survival as well as growth.
64	158	Removal of undesirable flavor characteristics. Modified flavor, aroma, texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: (survival of industrial processes, growth or storage in product formats, persistence in gut environment). Modified carbohydrate levels or	Homologue of pycB, encoding pyruvate carboxylase, which catalyzes a two-step reaction, involving the ATP-dependent carboxylation of the covalently attached biotin in the first step and the transfer of the carboxyl group to pyruvate to generate oxaloacetate in the second. The enzyme is involved in gluconeogenesis and amino acid biotransformations. Carbohydrate metabolism impacts on flavor, functionality and survival as well as growth.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		functional properties. Altered metabolic properties. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health) Improved fermentation properties or other industrially useful processes.	
66	160	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified lipid, glycolipid or free fatty acid levels or functional properties. Modified production of short chain fatty acids. Altered lipid metabolism. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health). Improved fermentation properties or other industrially useful processes.	Homologue of fox3, encoding a 3-oxoacyl-coenzyme A thiolase (EC 2.3.1.16) also called acetyl-CoA C-acyltransferase, which participates in the beta-oxidation of fatty acids. Expression of the FOX3 gene can be induced by oleate and repressed by glucose (Einerhand *et al., Mol. Cell. Biol.* 15:3405-3414, 1995). Free fatty acids are important in providing flavor-bearing compounds for dairy products such as cheese, and have a significant role in both flavor and texture. Used extensively in wide range of convenience foods. Short chain fatty acids are known to have a variety of health impacts.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
67	161	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	Homologue of YchH, encoding an acetyltransferase. Transfer of acetyl groups are important in regulation of metabolic pathways was well as co-factor production and can influence flavor and/or functionality.
68	162	Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Modified lipid, glycolipid or free fatty acid levels or functional properties. Modified production of short	Homologue of SC6F7, encoding a lipase. Lipases are involved in the breakdown of triglycerides, metabolism, growth, production of flavor compounds, and the release of free fatty acids. Can also catalyze esterification of glycerol to form mono, di- and triglycerides. Free fatty acids are important in providing flavor-bearing compounds for dairy products such as cheese, and have a significant role in both flavor and texture. Used extensively in wide range of convenience foods. Short chain fatty acids are known to have a variety of health impacts.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		chain fatty acids. Altered lipid metabolism. Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health). Improved fermentation properties or other industrially useful processes.	
69	163	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties. Improved fermentation properties or other industrially useful processes.	Homologue of yohH, a lipopolysaccharide synthesis protein. YohH is involved in techoic acid synthesis, and important for cell wall functions including adhesion, immune cell interaction, product texture.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
69	164	Altered cell wall or cell surface characteristics, structures or functions. Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved antimicrobial properties. Improved fermentation properties or other industrially useful processes.	Homologue of yohJ, encoding a lipopolysaccharide biosynthesis protein. YohJ is involved in techoic acid synthesis, and important for cell wall functions including adhesion, immune cell interaction and product texture.
70	165	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or	Homologue of pstS, encoding a phosphate-binding protein that is part of the phosphate specific transporter (Pst) in bacteria. Pst is a multisubunit system and belongs to the ABC superfamily of transporters (Novak *et al.*, *J Bacteriol.* 181:1126-1133, 1999). Intracellular phosphate levels influence survival of bacteria in

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	environmental stress conditions, and are involved in the stringent response.
71	166	Altered cell wall or cell surface characteristics, structures or functions. Production of bioactive or functional polypeptides. Modified adhesion to human or animal cells or cell lines. Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory,	Homologue of oppA, encoding an integral membrane protein of the oligopeptide transport system (Opp) of *Lactococcus lactis*. Opp proteins and the proteinase PrtP are important components of the proteolytic system. The Opp system belongs to the superfamily of ABC transporters and consists of five proteins: the integral membrane proteins OppB and OppC, the ATP-binding proteins OppD and OppF, and the OppA a receptor protein (Detmers *et al.*, *Proc. Natl. Acad. Sci. USA* 97:12487-12492, 2000). Important for the uptake and supply of amino acids to bacteria, and the resultant production of flavorful or functional amino-acid degradation products.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		anticancer, gut health). Improved fermentation properties or other industrially useful processes.	
71	167	Altered cell wall or cell surface characteristics, structures or functions. Production of bioactive or functional polypeptides. Modified adhesion to human or animal cells or cell lines. Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Improved fermentation properties or other industrially useful processes.	Homologue of oppB, encoding an integral membrane protein of the oligopeptide transport system (Opp) of *Lactococcus lactis*. Opp proteins and the proteinase PrtP are important components of the proteolytic system. The Opp system belongs to the superfamily of ABC transporters and consists of five proteins: the integral membrane proteins OppB and OppC, the ATP-binding proteins OppD and OppF, and the OppA a receptor protein (Detmers *et al., Proc. Natl. Acad. Sci. USA* 97:12487-12492, 2000). Important for the uptake and supply of amino acids to bacteria, and the resultant production of flavorful or functional amino-acid degradation products.
71	168	Altered cell wall or cell surface characteristics, structures or functions.	Homologue of OppC, encoding an integral membrane protein of the oligopeptide transport system (Opp)

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Production of bioactive or functional polypeptides. Modified adhesion to human or animal cells or cell lines. Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Improved fermentation properties or other industrially useful processes.	of *Lactococcus lactis*. Opp proteins and the proteinase PrtP are important components of the proteolytic system. The Opp system belongs to the superfamily of ABC transporters and consists of five proteins: the integral membrane proteins OppB and OppC, the ATP-binding proteins OppD and OppF, and the OppA a receptor protein (Detmers *et al., Proc. Natl. Acad. Sci. USA* 97:12487-12492, 2000). Important for the uptake and supply of amino acids to bacteria, and the resultant production of flavorful or functional amino-acid degradation products.
71	169	Altered cell wall or cell surface characteristics, structures or functions. Production of bioactive or functional polypeptides. Modified adhesion to human or animal cells or cell lines. Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors.	Homologue of OppF, encoding an integral membrane protein of the oligopeptide transport system (Opp) of *Lactococcus lactis*. Opp proteins and the proteinase PrtP are important components of the proteolytic system. The Opp system belongs to the superfamily of ABC transporters and consists of five proteins: the integral membrane proteins OppB and OppC, the ATP-binding proteins OppD and

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Improved fermentation properties or other industrially useful processes.	OppF, and the OppA a receptor protein (Detmers *et al., Proc. Natl. Acad. Sci. USA* 97:12487-12492, 2000). Important for the uptake and supply of amino acids to bacteria, and the resultant production of flavorful or functional amino-acid degradation products.
72	170	Removal of undesirable flavor characteristics. Modified flavor, aroma, texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: (survival of industrial processes, growth or storage in product formats, persistence in gut environment). Altered metabolic properties. Modified metabolism of sulphur-containing compounds.	Homologue of thdF, which is involved in the oxidation of derivatives of the sulphur-containing heterocycle thiophene and is induced during stationary phase. The thdF gene is subject to substantial catabolite repression by glucose and its expression is also greatly decreased in the absence of oxygen (Zabel *et al., Microbios.* 101:89-103, 2000).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Altered probiotic attributes. Organisms or materials with improved health properties (including immunoregulatory, anticancer, gut health).	
72	171	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	Homologue of gidA, encoding a glucose-inhibited division protein A, which is involved in cell division and in moderating translational fidelity (Kinscherf and Willis, *J. Bacteriol.* 184:2281-2286, 2002). Affects growth and viability in different growth environments.
73	172	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut	Homologue of poxB, encoding a pyruvate oxidase (EC 1.2.3.3), which decarboxylates pyruvate. The enzyme is a flavoprotein (FAD) requiring thiamine diphosphate and is important for aerobic growth and survival in aerobic conditions. Carbohydrate metabolism impacts on flavor, functionality and survival as well as growth.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	
74	173	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	Homologue of gltD, encoding a glutamate synthase (EC 1.4.1.13), which catalyzes the reductive transfer of the amide group of glutamine to the keto position of 2-oxoglutarate to yield two molecules of glutamate. The resulting glutamine and glutamate serve as nitrogen donors in the biosynthesis of various nitrogen-containing compounds. This pathway is involved in the integration of carbon and nitrogen assimilations. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
75	174	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein	Homologue of dhfR, encoding a dihydrofolate reductase (EC 1.5.1.3), which catalyzes the essential step for *de novo* glycine and purine synthesis, DNA precursor synthesis, and for the conversion of dUMP to dTMP. Involved in folate production, which has major health impacts and also detoxifies some chemotherapeutic

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Increased folate production. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Protection of intestinal cells from toxic compounds. Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	drugs and other cytotoxic compounds.
77	176	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes.	Homologue of trpA, encoding the tryptophan synthase alpha chain (EC 4.2.1.20). TrpA catalyzes the formation of indole from the cleavage of 3-indolyl-D-glyceraldehyde 3'-phosphate. Seven structural genes are required for tryptophan biosynthesis: trpABCDEFG. TrpA encodes the tryptophan synthase alpha chain (EC 4.2.1.20) Tryptophan is important for flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	
77	177	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	Homologue of trpB, encoding tryptophan synthase beta chain (EC 4.2.1.20). TrpB catalyzes the condensation of indole to a serine-derived aminoacrylate moiety bound to pyridoxal phosphate. Seven structural genes are required for tryptophan biosynthesis: trpABCDEFG. TrpB encodes the tryptophan synthase beta chain (EC 4.2.1.20). Tryptophan is important for flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al.*, *Appl. Environ. Microbiol.* 65:4873-4880, 1999).
77	178	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other	Homologue of trpC, encoding the bifunctional enzyme phosphoribosylanthranilate isomerase (EC 5.3.1.24) -indoleglycerol phosphate synthetase (EC 4.1.1.48). TrpC catalyzes the ring closure of 1-(2-carboxyphenylamino)-1-deoxyribulose 5'-phosphate to indoleglycerol phosphate, the fifth step in the pathway of tryptophan biosynthesis from chorismate. Seven structural genes are required for

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	tryptophan biosynthesis: trpABCDEFG. Tryptophan is important for flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
77	179	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	Homologue of trpD, encoding phosphoribosyl anthranilate transferase (EC 2.4.2.18),. TrpD catalyzes the reaction N-(5-phospho-D-ribosyl)-anthranilate + diphosphate = anthranilate + 5-phospho-a-D-ribose 1-diphosphate. Seven structural genes are required for tryptophan biosynthesis: trpABCDEFG. Tryptophan is important for flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
77	180	Altered amino acid metabolism. Removal of undesirable flavor characteristics. Production of desirable flavors.	Homologue of trpF, encoding phosphoribosylanthranilate isomerase (EC 5.3.1.24). TrpF catalyzes the conversion of N-(5'-

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance.	diphosphoribosyl)anthranilate to 1-(o-carboxyphenylamino)-1-deoxyribulose 5 phosphate. Seven structural genes are required for tryptophan biosynthesis: trpABCDEFG. Tryptophan is important for flavor development. Amino acid degradation products greatly contribute to flavor or to off-flavors (Rijnen *et al., Appl. Environ. Microbiol.* 65:4873-4880, 1999).
78	181	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	Homologue of purine nucleoside phosphorylase (PNP) (EC 2.4.2.1). PNP catalyzes the reversible phosphorolysis of (2'-deoxy)purine ribonucleosides to free base and (2'-deoxy)ribose-1-phosphate and has a metabolic role in purine salvage. Intracellular phosphate levels influence survival of bacteria in environmental stress conditions, and are involved in the stringent response.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
79	182	Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Altered survival characteristics: survival of industrial processes, growth or storage in product formats, persistence in gut environment. Altered viability in response to stress conditions. Altered metabolic properties or regulation of metabolic pathways. Altered probiotic attributes.	Homologue to relA. RelA plays a role in synthesis and degradation of the highly phosphorylated guanosine nucleotides (p)ppGp. Intracellular phosphate levels influence survival of bacteria in environmental stress conditions, and are involved in the stringent response.
80	183	Altered cell wall or cell surface characteristics, structures or functions. Improved antimicrobial properties Modified adhesion to human or animal cells or cell lines. Production of desirable flavors. Modified flavor, aroma and/or texture attributes. Construction of genetic vectors for controlled expression of RNA and/or protein, fusion protein production, genetic modification, mutagenesis amplification of genetic material or for other genetic or protein manipulations. Altered survival characteristics: survival of industrial processes,	Homologue of lysostaphin, an antimicrobial immunity factor of *Staphylococcus simulans* biovar *staphylolyticus* active against *Staphylococcus aureas*. Lysostaphin is currently being investigated for use against mastitis in dairy cattle, caused by *Staphylococcus aureus* (Kerr *et al.*, *Nat. Biotechnol.* 19:66-70, 2001). The gene contains the conserved motif GPHLHF, which is also present in several secreted peptidases. Lysostaphin has utility as an antimicrobial for human and veterinary use.

SEQ ID NO: DNA	SEQ ID NO: Polypeptide	Utility	Description
		growth or storage in product formats, persistence in gut environment. Altered metabolic properties. Altered probiotic attributes. Modified health properties (including immunoregulatory, anticancer, gut health). Modified antibiotic resistance. Improved fermentation properties or other industrially useful processes.	

Isolated polynucleotides of the present invention include the polynucleotides identified herein as SEQ ID NOS: 1-80; isolated polynucleotides comprising a polynucleotide sequence selected from the group consisting of SEQ ID NOS: 1-80; isolated polynucleotides comprising at least a specified number of contiguous residues (x-mers) of any of the polynucleotides identified as SEQ ID NOS: 1-80; isolated polynucleotides comprising a polynucleotide sequence that is complementary to any of the above polynucleotides; isolated polynucleotides comprising a polynucleotide sequence that is a reverse sequence or a reverse complement of any of the above polynucleotides; antisense sequences corresponding to any of the above polynucleotides; and variants of any of the above polynucleotides, as that term is described in this specification.

The word "polynucleotide(s)," as used herein, means a single or double stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including mRNA molecules, both sense and antisense strands of DNA and RNA molecules, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. A polynucleotide of the present invention may be an entire gene, or any portion thereof. A gene is a DNA sequence which codes for a functional protein or RNA molecule. Operable antisense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all operable antisense fragments. Antisense polynucleotides and techniques involving antisense

polynucleotides are well known in the art and are described, for example, in Robinson-Benion, et al., "Antisense techniques," *Methods in Enzymol.* 254(23): 363-375, 1995; and Kawasaki, *et al., Artific. Organs* 20 (8): 836-848, 1996.

The definitions of the terms "complement," "reverse complement," and "reverse sequence," as used herein, are best illustrated by the following examples. For the sequence 5' AGGACC 3', the complement, reverse complement, and reverse sequences are as follows:

complement	3' TCCTGG 5'
reverse complement	3' GGTCCT 5'
reverse sequence	5' CCAGGA 3'

Identification of genomic DNA and heterologous species DNA can be accomplished by standard DNA/DNA hybridization techniques, under appropriately stringent conditions, using all or part of a DNA sequence as a probe to screen an appropriate library. Alternatively, PCR techniques using oligonucleotide primers that are designed based on known DNA and protein sequences can be used to amplify and identify other identical or similar DNA sequences. Synthetic DNA corresponding to the identified sequences or variants thereof may be produced by conventional synthesis methods. All of the polynucleotides described herein are isolated and purified, as those terms are commonly used in the art.

The polynucleotides identified as SEQ ID NOS: 1-80 contain open reading frames ("ORFs"), or partial open reading frames, encoding polypeptides. Additionally, polynucleotides identified as SEQ ID NOS: 1-80 may contain non-coding sequences such as promoters and terminators that may be useful as control elements. Additionally, open reading frames encoding polypeptides may be identified in extended or full-length sequences corresponding to the sequences set out as SEQ ID NOS: 81-183. Open reading frames may be identified using techniques that are well known in the art. These techniques include, for example, analysis for the location of known start and stop codons, most likely reading frame identification based on codon frequencies, similarity to known bacterial expressed genes, etc. Tools and software suitable for ORF analysis include GeneWise (The Sanger Center, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom), Diogenes (Computational Biology Centers, University of Minnesota, Academic Health Center, UMHG Box 43 Minneapolis MN 55455), and GRAIL (Informatics Group, Oak Ridge National Laboratories, Oak Ridge, Tennessee, TN).

Open reading frames and portions of open reading frames may be identified in the polynucleotides of the present invention. Once a partial open reading frame is identified, the polynucleotide may be extended in the area of the partial open reading frame using techniques that are well known in the art until the polynucleotide for the full open reading frame is identified. Thus, polynucleotides and open reading frames encoding polypeptides may be identified using the polynucleotides of the present invention.

Once open reading frames are identified in the polynucleotides of the present invention, the open reading frames may be isolated and/or synthesized. Expressible genetic constructs comprising the open reading frames and suitable promoters, initiators, terminators, etc., which are well known in the art, may then be constructed. Such genetic constructs may be introduced into a host cell to express the polypeptide encoded by the open reading frame. Suitable host cells may include various prokaryotic and eukaryotic cells. *In vitro* expression of polypeptides is also possible, as well known in the art.

As used herein, the term "oligonucleotide" refers to a relatively short segment of a polynucleotide sequence, generally comprising between 6 and 60 nucleotides, and comprehends both probes for use in hybridization assays and primers for use in the amplification of DNA by polymerase chain reaction.

As used herein, the term "*x*-mer," with reference to a specific value of "*x*," refers to a polynucleotide comprising at least a specified number ("*x*") of contiguous residues of any of the polynucleotides identified as SEQ ID NOS: 1-80. The value of *x* may be from about 20 to about 600, depending upon the specific sequence.

In another aspect, the present invention provides isolated polypeptides encoded, or partially encoded, by the above polynucleotides. In specific embodiments, such polypeptides comprise a sequence selected from the group consisting of SEQ ID NO: 81-183, and variants thereof. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full-length proteins, wherein the amino acid residues are linked by covalent peptide bonds. The term "polypeptide encoded by a polynucleotide" as used herein, includes polypeptides encoded by a polynucleotide which comprises an isolated polynucleotide sequence or variant provided herein. Polypeptides of the present invention may be naturally purified products, or may be produced partially or wholly using recombinant techniques. Such

polypeptides may be glycosylated with bacterial, fungal, mammalian or other eukaryotic carbohydrates or may be non-glycosylated.

Polypeptides of the present invention may be produced recombinantly by inserting a polynucleotide that encodes the polypeptide into an expression vector and expressing the polypeptide in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a polypeptide encoding a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *Escherichia coli*, *Lactococcus lactis*, *Lactobacillus*, insect, yeast or a mammalian cell line such as COS or CHO. The polynucleotide(s) expressed in this manner may encode naturally occurring polypeptides, portions of naturally occurring polypeptides, or other variants thereof.

In a related aspect, polypeptides are provided that comprise at least a functional portion of a polypeptide having an amino acid sequence encoded by a polynucleotide of the present invention. As used herein, a "functional portion" of a polypeptide is that portion which contains the active site essential for affecting the function of the polypeptide, for example, the portion of the molecule that is capable of binding one or more reactants. The active site may be made up of separate portions present on one or more polypeptide chains and will generally exhibit high binding affinity.

Functional portions of a polypeptide may be identified by first preparing fragments of the polypeptide by either chemical or enzymatic digestion of the polypeptide, or by mutation analysis of the polynucleotide that encodes the polypeptide and subsequent expression of the resulting mutant polypeptides. The polypeptide fragments or mutant polypeptides are then tested to determine which portions retain biological activity, using, for example, the representative assays provided below.

Portions and other variants of the inventive polypeptides may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques that are well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-

phase synthesis method, where amino acids are sequentially added to a growing amino acid chain (*See* Merrifield, *J. Am. Chem. Soc.* 85:2149-2154, 1963). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied Biosystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions. Variants of a native polypeptide may be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (Kunkel, *Proc. Natl. Acad. Sci. USA* 82: 488-492, 1985). Sections of DNA sequences may also be removed using standard techniques to permit preparation of truncated polypeptides.

In general, the polypeptides disclosed herein are prepared in an isolated, substantially pure form. Preferably, the polypeptides are at least about 80% pure; more preferably at least about 90% pure; and most preferably at least about 99% pure.

As used herein, the term "variant" comprehends polynucleotide or polypeptide sequences different from the specifically identified sequences, wherein one or more nucleotides or amino acid residues is deleted, substituted, or added. Variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant polynucleotide sequences preferably exhibit at least 60%, more preferably at least 75%, more preferably yet at least 90%, and most preferably at least 95% identity to a sequence of the present invention. Variant polypeptide sequences preferably exhibit at least 60%, more preferably at least 75%, more preferably yet at least 90%, and most preferably at least 95% identity to a sequence of the present invention. The percentage identity is determined by aligning the two sequences to be compared as described below, determining the number of identical residues in the aligned portion, dividing that number by the total number of residues in the inventive (queried) sequence, and multiplying the result by 100.

Polynucleotide and polypeptide sequences may be aligned, and the percentage of identical residues in a specified region may be determined against another polynucleotide or polypeptide, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences are the BLASTN and FASTA algorithms. Polynucleotides may also be analyzed using the BLASTX algorithm, which compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database. The percentage identity of polypeptide sequences

may be examined using the BLASTP algorithm. The BLASTN, BLASTX and BLASTP programs are available on the NCBI anonymous FTP server and from the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894, USA. The BLASTN algorithm Version 2.0.4 [Feb-24-1998], Version 2.0.6 [Sept-16-1998] and Version 2.0.11 [Jan-20-2000], set to the parameters described below, is preferred for use in the determination of polynucleotide variants according to the present invention. The BLASTP algorithm, set to the parameters described below, is preferred for use in the determination of polypeptide variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN, BLASTP and BLASTX, is described in the publication of Altschul *et al., Nucleic Acids Res.* 25: 3389-3402, 1997.

The computer algorithm FASTA is available on the Internet and from the University of Virginia by contacting David Hudson, Vice Provost for Research, University of Virginia, P.O. Box 9025, Charlottesville, VA 22906-9025, USA. FASTA Version 2.0u4 [February 1996], set to the default parameters described in the documentation and distributed with the algorithm, may be used in the determination of variants according to the present invention. The use of the FASTA algorithm is described in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444-2448, 1988; and Pearson , *Methods in Enzymol.* 183: 63-98, 1990.

The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to the E values and percentage identity for polynucleotide sequences: Unix running command: blastall -p blastn -d embldb -e 10 –G0 -E0 -r 1 -v 30 -b 30 -i queryseq –o results; the parameters are: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -r Reward for a nucleotide match (BLASTN only) [Integer]; -v Number of one-line descriptions (V) [Integer]; -b Number of alignments to show (B) [Integer]; -i Query File [File In]; and -o BLAST report Output File [File Out] Optional.

The following running parameters are preferred for determination of alignments and similarities using BLASTP that contribute to the E values and percentage identity of polypeptide sequences: blastall –p blastp –d swissprottrembledb –e 10 -G 0 -E 0 –v 30 –b 30 –i queryseq –o results; the parameters are: -p Program Name [String]; -d Database [String]; -e Expectation

value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -v Number of one-line descriptions (v) [Integer]; -b Number of alignments to show (b) [Integer]; -I Query File [File In]; -o BLAST report Output File [File Out] Optional. The "hits" to one or more database sequences by a queried sequence produced by BLASTN, FASTA, BLASTP or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

The BLASTN, FASTA, and BLASTP algorithms also produce "Expect" values for alignments. The Expect value (E) indicates the number of hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database, such as the preferred EMBL database, indicates true similarity. For example, an E value of 0.1 assigned to a polynucleotide hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion of the sequence with a similar score simply by chance. By this criterion, the aligned and matched portions of the polynucleotide sequences then have a probability of 90% of being the same. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN or FASTA algorithm.

According to one embodiment, "variant" polynucleotides and polypeptides, with reference to each of the polynucleotides and polypeptides of the present invention, preferably comprise sequences producing an E value of 0.01 or less when compared to the polynucleotide or polypeptide of the present invention. That is, a variant polynucleotide or polypeptide is any sequence that has at least a 99% probability of being the same as the polynucleotide or polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTN, FASTA, or BLASTP algorithms set at parameters described above. According to a preferred embodiment, a variant polynucleotide is a sequence having the same number or fewer nucleic acids than a polynucleotide of the present invention that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of

0.01 or less using the BLASTN or FASTA algorithms set at parameters described above. Similarly, according to a preferred embodiment, a variant polypeptide is a sequence having the same number or fewer amino acids than a polypeptide of the present invention that has at least a 99% probability of being the same as a polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTP algorithm set at the parameters described above.

As noted above, the percentage identity is determined by aligning sequences using one of the BLASTN, FASTA, or BLASTP algorithms, set at the running parameters described above, and identifying the number of identical nucleic or amino acids over the aligned portions; dividing the number of identical nucleic or amino acids by the total number of nucleic or amino acids of the polynucleotide or polypeptide sequence of the present invention; and then multiplying by 100 to determine the percentage identity. For example, a polynucleotide of the present invention having 220 nucleic acids has a hit to a polynucleotide sequence in the EMBL database having 520 nucleic acids over a stretch of 23 nucleotides in the alignment produced by the BLASTN algorithm using the parameters described above. The 23 nucleotide hit includes 21 identical nucleotides, one gap and one different nucleotide. The percentage identity of the polynucleotide of the present invention to the hit in the EMBL library is thus 21/220 times 100, or 9.5%. The polynucleotide sequence in the EMBL database is thus not a variant of a polynucleotide of the present invention.

In addition to having a specified percentage identity to an inventive polynucleotide or polypeptide sequence, variant polynucleotides and polypeptides preferably have additional structure and/or functional features in common with the inventive polynucleotide or polypeptide. Polypeptides having a specified degree of identity to a polypeptide of the present invention share a high degree of similarity in their primary structure and have substantially similar functional properties. In addition to sharing a high degree of similarity in their primary structure to polynucleotides of the present invention, polynucleotides having a specified degree of identity to, or capable of hybridizing to an inventive polynucleotide preferably have at least one of the following features: (i) they contain an open reading frame or partial open reading frame encoding a polypeptide having substantially the same functional properties as the polypeptide encoded by the inventive polynucleotide; or (ii) they contain identifiable domains in common.

Alternatively, variant polynucleotides of the present invention hybridize to the polynucleotide sequences recited in SEQ ID NOS: 1-80, or complements, reverse sequences, or reverse complements of those sequences under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65° C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65°C.

The present invention also encompasses polynucleotides that differ from the disclosed sequences but that, as a consequence of the discrepancy of the genetic code, encode a polypeptide having similar enzymatic activity as a polypeptide encoded by a polynucleotide of the present invention. Thus, polynucleotides comprising sequences that differ from the polynucleotide sequences recited in SEQ ID NOS: 1-80, or complements, reverse sequences, or reverse complements of those sequences as a result of conservative substitutions are encompassed within the present invention. Additionally, polynucleotides comprising sequences that differ from the inventive polynucleotide sequences or complements, reverse complements, or reverse sequences as a result of deletions and/or insertions totaling less than 10% of the total sequence length are also contemplated by and encompassed within the present invention. Similarly, polypeptides comprising sequences that differ from the inventive polypeptide sequences as a result of amino acid substitutions, insertions, and/or deletions totaling less than 10% of the total sequence length are contemplated by and encompassed within the present invention, provided the variant polypeptide has similar activity to the inventive polypeptide.

The polynucleotides of the present invention may be isolated from various libraries, or may be synthesized using techniques that are well known in the art. The polynucleotides may be synthesized, for example, using automated oligonucleotide synthesizers (*e.g.*, Beckman Oligo 1000M DNA Synthesizer) to obtain polynucleotide segments of up to 50 or more nucleic acids. A plurality of such polynucleotide segments may then be ligated using standard DNA manipulation techniques that are well known in the art of molecular biology. One conventional and exemplary polynucleotide synthesis technique involves synthesis of a single stranded polynucleotide segment having, for example, 80 nucleic acids, and hybridizing that segment to a synthesized complementary 85 nucleic acid segment to produce a 5-nucleotide overhang. The next segment may then be synthesized in a similar fashion, with a 5-nucleotide overhang on the

opposite strand. The "sticky" ends ensure proper ligation when the two portions are hybridized. In this way, a complete polynucleotide of the present invention may be synthesized entirely *in vitro*.

Certain of the polynucleotides identified as SEQ ID NOS: 1-80 are generally referred to as "partial" sequences, in that they may not represent the full coding portion of a gene encoding a naturally occurring polypeptide. The partial polynucleotide sequences disclosed herein may be employed to obtain the corresponding full-length genes for various species and organisms by, for example, screening DNA expression libraries using hybridization probes based on the polynucleotides of the present invention, or using PCR amplification with primers based upon the polynucleotides of the present invention. In this way one can, using methods well known in the art, extend a polynucleotide of the present invention upstream and downstream of the corresponding DNA, as well as identify the corresponding mRNA and genomic DNA, including the promoter and enhancer regions, of the complete gene. The present invention thus comprehends isolated polynucleotides comprising a sequence identified in SEQ ID NOS: 1-80, or a variant of one of the specified sequences, that encode a functional polypeptide, including full length genes. Such extended polynucleotides may have a length of from about 50 to about 4,000 nucleic acids or base pairs, and preferably have a length of less than about 4,000 nucleic acids or base pairs, more preferably yet a length of less than about 3,000 nucleic acids or base pairs, more preferably yet a length of less than about 2,000 nucleic acids or base pairs. Under some circumstances, extended polynucleotides of the present invention may have a length of less than about 1,800 nucleic acids or base pairs, preferably less than about 1,600 nucleic acids or base pairs, more preferably less than about 1,400 nucleic acids or base pairs, more preferably yet less than about 1,200 nucleic acids or base pairs, and most preferably less than about 1,000 nucleic acids or base pairs.

Polynucleotides of the present invention comprehend polynucleotides comprising at least a specified number of contiguous residues (x-mers) of any of the polynucleotides identified as SEQ ID NOS: 1-80 or their variants. According to preferred embodiments, the value of x is preferably at least 20, more preferably at least 40, more preferably yet at least 60, and most preferably at least 80. Thus, polynucleotides of the present invention include polynucleotides comprising a 20-mer, a 40-mer, a 60-mer, an 80-mer, a 100-mer, a 120-mer, a 150-mer, a 180-

mer, a 220-mer a 250-mer, or a 300-mer, 400-mer, 500-mer or 600-mer of a polynucleotide identified as SEQ ID NOS: 1-80 or a variant of one of the polynucleotides identified as SEQ ID NOS: 1-80.

Oligonucleotide probes and primers complementary to and/or corresponding to SEQ ID NOS: 1-80, and variants of those sequences, are also comprehended by the present invention. Such oligonucleotide probes and primers are substantially complementary to the polynucleotide of interest. An oligonucleotide probe or primer is described as "corresponding to" a polynucleotide of the present invention, including one of the sequences set out as SEQ ID NOS: 1-80 or a variant, if the oligonucleotide probe or primer, or its complement, is contained within one of the sequences set out as SEQ ID NOS: 1-80 or a variant of one of the specified sequences.

Two single stranded sequences are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared, with the appropriate nucleotide insertions and/or deletions, pair with at least 80%, preferably at least 90% to 95%, and more preferably at least 98% to 100%, of the nucleotides of the other strand. Alternatively, substantial complementarity exists when a first DNA strand will selectively hybridize to a second DNA strand under stringent hybridization conditions. Stringent hybridization conditions for determining complementarity include salt conditions of less than about 1 M, more usually less than about 500 mM and preferably less than about 200 mM. Hybridization temperatures can be as low as 5°C, but are generally greater than about 22°C, more preferably greater than about 30°C and most preferably greater than about 37°C. Longer DNA fragments may require higher hybridization temperatures for specific hybridization. Since the stringency of hybridization may be affected by other factors such as probe composition, presence of organic solvents and extent of base mismatching, the combination of parameters is more important than the absolute measure of any one alone. DNA-DNA hybridization studies may performed using either genomic DNA or DNA derived by preparing cDNA from the RNA present in a sample to be tested.

In addition to DNA-DNA hybridization, DNA-RNA or RNA-RNA hybridization assays are also possible. In the first case, the mRNA from expressed genes would then be detected instead of genomic DNA or cDNA derived from mRNA of the sample. In the second case, RNA

probes could be used. In addition, artificial analogs of DNA hybridizing specifically to target sequences could also be used.

In specific embodiments, the oligonucleotide probes and/or primers comprise at least about 6 contiguous residues, more preferably at least about 10 contiguous residues, and most preferably at least about 20 contiguous residues complementary to a polynucleotide sequence of the present invention. Probes and primers of the present invention may be from about 8 to 100 base pairs in length or, preferably from about 10 to 50 base pairs in length or, more preferably from about 15 to 40 base pairs in length. The primers and probes may be readily selected using procedures well known in the art, taking into account DNA-DNA hybridization stringencies, annealing and melting temperatures, potential for formation of loops and other factors, which are well known in the art. Tools and software suitable for designing probes, and especially for designing PCR primers, are available from Premier Biosoft International, 3786 Corina Way, Palo Alto, CA 94303-4504. Preferred techniques for designing PCR primers are also disclosed in Dieffenbach and Dyksler, *PCR primer: a laboratory manual*, CSHL Press: Cold Spring Harbor, NY, 1995.

A plurality of oligonucleotide probes or primers corresponding to a polynucleotide of the present invention may be provided in a kit form. Such kits generally comprise multiple DNA or oligonucleotide probes, each probe being specific for a polynucleotide sequence. Kits of the present invention may comprise one or more probes or primers corresponding to a polynucleotide of the present invention, including a polynucleotide sequence identified in SEQ ID NOS: 1-80.

In one embodiment useful for high-throughput assays, the oligonucleotide probe kits of the present invention comprise multiple probes in an array format, wherein each probe is immobilized in a predefined, spatially addressable location on the surface of a solid substrate. Array formats which may be usefully employed in the present invention are disclosed, for example, in U.S. Patents No. 5,412,087, 5,545,531, and PCT Publication No. WO 95/00530, the disclosures of which are hereby incorporated by reference.

Oligonucleotide probes for use in the present invention may be constructed synthetically prior to immobilization on an array, using techniques well known in the art (*See*, for example, Gait, ed., *Oligonucleotide synthesis a practical approach*, IRL Press: Oxford, England, 1984).

Automated equipment for the synthesis of oligonucleotides is available commercially from such companies as Perkin Elmer/Applied Biosystems Division (Foster City, CA) and may be operated according to the manufacturer's instructions. Alternatively, the probes may be constructed directly on the surface of the array using techniques taught, for example, in PCT Publication No. WO 95/00530.

The solid substrate and the surface thereof preferably form a rigid support and are generally formed from the same material. Examples of materials from which the solid substrate may be constructed include polymers, plastics, resins, membranes, polysaccharides, silica or silica-based materials, carbon, metals and inorganic glasses. Synthetically prepared probes may be immobilized on the surface of the solid substrate using techniques well known in the art, such as those disclosed in U.S. Patent No. 5,412,087.

In one such technique, compounds having protected functional groups, such as thiols protected with photochemically removable protecting groups, are attached to the surface of the substrate. Selected regions of the surface are then irradiated with a light source, preferably a laser, to provide reactive thiol groups. This irradiation step is generally performed using a mask having apertures at predefined locations using photolithographic techniques well known in the art of semiconductors. The reactive thiol groups are then incubated with the oligonucleotide probe to be immobilized. The precise conditions for incubation, such as temperature, time and pH, depend on the specific probe and can be easily determined by one of skill in the art. The surface of the substrate is washed free of unbound probe and the irradiation step is repeated using a second mask having a different pattern of apertures. The surface is subsequently incubated with a second, different, probe. Each oligonucleotide probe is typically immobilized in a discrete area of less than about 1 mm^2. Preferably each discrete area is less than about 10,000 mm^2, more preferably less than about 100 mm^2. In this manner, a multitude of oligonucleotide probes may be immobilized at predefined locations on the array.

The resulting array may be employed to screen for differences in organisms or samples or products containing genetic material as follows. Genomic or cDNA libraries are prepared using techniques well known in the art. The resulting target DNA is then labeled with a suitable marker, such as a radiolabel, chromophore, fluorophore or chemiluminescent agent, using

protocols well known for those skilled in the art. A solution of the labeled target DNA is contacted with the surface of the array and incubated for a suitable period of time.

The surface of the array is then washed free of unbound target DNA and the probes to which the target DNA hybridized are determined by identifying those regions of the array to which the markers are attached. When the marker is a radiolabel, such as ^{32}P, autoradiography is employed as the detection method. In one embodiment, the marker is a fluorophore, such as fluorescein, and the location of bound target DNA is determined by means of fluorescence spectroscopy. Automated equipment for use in fluorescence scanning of oligonucleotide probe arrays is available from Affymetrix, Inc. (Santa Clara, CA) and may be operated according to the manufacturer's instructions. Such equipment may be employed to determine the intensity of fluorescence at each predefined location on the array, thereby providing a measure of the amount of target DNA bound at each location. Such an assay would be able to indicate not only the absence and presence of the marker probe in the target, but also the quantitative amount as well.

The significance of such high-throughput screening system is apparent for applications such as microbial selection and quality control operations in which there is a need to identify large numbers of samples or products for unwanted materials, to identify microbes or samples or products containing microbial material for quarantine purposes, etc., or to ascertain the true origin of samples or products containing microbes. Screening for the presence or absence of polynucleotides of the present invention used as identifiers for tagging microbes and microbial products can be valuable for later detecting the genetic composition of food, fermentation and industrial microbes or microbes in human or animal digestive system after consumption of probiotics, etc.

In this manner, oligonucleotide probe kits of the present invention may be employed to examine the presence/absence (or relative amounts in case of mixtures) of polynucleotides in different samples or products containing different materials rapidly and in a cost-effective manner. Examples of microbial species which may be examined using the present invention, include lactic acid bacteria, such as *Lactobacillus rhamnosus*, and other microbial species.

Another aspect of the present invention involves collections of a plurality of polynucleotides of the present invention. A collection of a plurality of the polynucleotides of the present invention, particularly the polynucleotides identified as SEQ ID NOS: 1-80, may be

recorded and/or stored on a storage medium and subsequently accessed for purposes of analysis, comparison, etc. Suitable storage media include magnetic media such as magnetic diskettes, magnetic tapes, CD-ROM storage media, optical storage media, and the like. Suitable storage media and methods for recording and storing information, as well as accessing information such as polynucleotide sequences recorded on such media, are well known in the art. The polynucleotide information stored on the storage medium is preferably computer-readable and may be used for analysis and comparison of the polynucleotide information.

Another aspect of the present invention thus involves storage medium on which are recorded a collection of the polynucleotides of the present invention, particularly a collection of the polynucleotides identified as SEQ ID NOS: 1-80. According to one embodiment, the storage medium includes a collection of at least 20, preferably at least 50, more preferably at least 100, and most preferably at least 200 of the polynucleotides of the present invention, preferably the polynucleotides identified as SEQ ID NOS: 1-80, including variants of those polynucleotides.

Another aspect of the present invention involves a combination of polynucleotides, the combination containing at least 5, preferably at least 10, more preferably at least 20, and most preferably at least 50 different polynucleotides of the present invention, including polynucleotides selected from SEQ ID NOS: 1-80, and variants of these polynucleotides.

In another aspect, the present invention provides genetic constructs comprising, in the 5'-3' direction, a gene promoter sequence and an open reading frame coding for at least a functional portion of a polypeptide encoded by a polynucleotide of the present invention. In certain embodiments, the genetic constructs of the present invention also comprise a gene termination sequence. The open reading frame may be oriented in either a sense or antisense direction. Genetic constructs comprising a non-coding region of a gene coding for a polypeptide encoded by an inventive polynucleotide or a nucleotide sequence complementary to a non-coding region, together with a gene promoter sequence, are also provided. A terminator sequence may form part of this construct. Preferably, the gene promoter and termination sequences are functional in a host organism. More preferably, the gene promoter and termination sequences are common to those of the polynucleotide being introduced. The genetic construct may further include a marker for the identification of transformed cells.

Techniques for operatively linking the components of the genetic constructs are well known in the art and include the use of synthetic linkers containing one or more restriction endonuclease sites as described, for example, by Sambrook *et al.*, in *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratories Press: Cold Spring Harbor, NY, 1989. The genetic constructs of the present invention may be linked to a vector having at least one replication system, for example, *E. coli*, whereby after each manipulation, the resulting construct can be cloned and sequenced and the correctness of the manipulation determined.

Transgenic microbial cells comprising the genetic constructs of the present invention are also provided by the present invention, together with microbes comprising such transgenic cells, products and progeny of such microbes, and materials including such microbes. Techniques for stably incorporating genetic constructs into the genome of target microbes, such as *Lactobacillus* species, *Lactococcus lactis* or *E. coli,* are well known in the art of bacterial transformation and are exemplified by the transformation of *E. coli* for sequencing described in Example 1.

Transgenic non-microbial cells comprising the genetic constructs of the present invention are also provided, together with organisms comprising such transgenic cells, and products and progeny of such organisms. Genetic constructs of the present invention may be stably incorporated into the genomes of non-microbial target organisms, such as fungi, using techniques well known in the art.

In preferred embodiments, the genetic constructs of the present invention are employed to transform microbes used in the production of food products, ingredients, processing aids, additives or supplements and for the production of microbial products for pharmaceutical uses, particularly for modulating immune system function and immunological effects, and in the production of chemoprotectants providing beneficial effects, probiotics and health supplements. The inventive genetic constructs may also be employed to transform bacteria that are used to produce enzymes or substances such as polysaccharides, flavor compounds and bioactive substances, and to enhance resistance to industrial processes such as drying and to adverse stimuli in the human digestive system. The genes involved in antibiotic production, and phage uptake and resistance in *Lactobacillus rhamnosus* are considered to be especially useful. The target microbe to be used for transformation with one or more polynucleotides or genetic constructs of the present invention is preferably selected from the group consisting of bacterial

genera *Lactococcus, Lactobacillus, Streptococcus, Oenococcus, Lactosphaera, Trichococcus, Pediococcus* and others potentially useful in various fermentation industries and is most preferably selected from the group consisting of the following *Lactobacillus* species: *Lactobacillus acetotolerans, Lactobacillus acidophilus, Lactobacillus agilis, Lactobacillus alimentarius, Lactobacillus amylolyticus, Lactobacillus amylophilus, Lactobacillus amylovorus, Lactobacillus animalis, Lactobacillus arizonae, Lactobacillus aviarius, Lactobacillus bavaricus, Lactobacillus bifermentans, Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus collinoides, Lactobacillus coryniformis, Lactobacillus crispatus, Lactobacillus curvatus, Lactobacillus delbrueckii, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus delbrueckii* subsp. *lactis, Lactobacillus farciminis, Lactobacillus fermentum, Lactobacillus fructivorans, Lactobacillus gallinarum, Lactobacillus gasseri, Lactobacillus graminis, Lactobacillus hamsteri, Lactobacillus helveticus, Lactobacillus helveticus* subsp. *jugurti, Lactobacillus hetero, Lactobacillus hilgardii, Lactobacillus homohiochii, Lactobacillus japonicus, Lactobacillus johnsonii, Lactobacillus kefiri, Lactobacillus lactis, Lactobacillus leichmannii, Lactobacillus lindneri, Lactobacillus mali, Lactobacillus maltaromicus, Lactobacillus manihotivorans, Lactobacillus mucosae, Lactobacillus murinus, Lactobacillus oris, Lactobacillus panis, Lactobacillus paracasei, Lactobacillus paracasei* subsp. *pseudoplantarum, Lactobacillus paraplantarum, Lactobacillus pentosus, Lactobacillus plantarum, Lactobacillus pontis, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus ruminis, Lactobacillus sake, Lactobacillus salivarius, Lactobacillus salivarius* subsp. *salicinius, Lactobacillus salivarius* subsp. *salivarius, Lactobacillus sanfranciscensis, Lactobacillus sharpeae, Lactobacillus thermophilus, Lactobacillus vaginalis, Lactobacillus vermiforme,* and *Lactobacillus zeae.*

In yet a further aspect, the present invention provides methods for modifying the concentration, composition and/or activity of a polypeptide in a host organism, such as a microbe, comprising stably incorporating a genetic construct of the present invention into the genome of the host organism by transforming the host organism with such a genetic construct. The genetic constructs of the present invention may be used to transform a variety of organisms including plants, such as monocotyledonous angiosperms (*e.g.*, grasses, corn, grains, oat, wheat and barley); dicotyledonous angiosperms (*e.g.*, *Arabidopsis*, tobacco, legumes, alfalfa, oaks,

eucalyptus, maple); gymnosperms, (*e.g.*, Scots pine (Aronen, *Finnish Forest Res. Papers*, Vol. 595, 1996); white spruce (Ellis *et al.*, *Biotechnology* 11:84-89, 1993); larch (Huang, *et al.*, *In Vitro Cell* 27:201-207, 1991); and any kind of plant amenable to genetic engineering.

Thus, in yet another aspect, transgenic plant cells comprising the genetic constructs of the present invention are provided, together with plants comprising such transgenic cells, and fruits, seeds, products and progeny of such plants. Techniques for stably incorporating genetic constructs into the genome of target organisms, such as plants, are well known in the art and include *Agrobacterium tumefaciens* mediated introduction, electroporation, protoplast fusion, injection into reproductive organs, injection into immature embryos, high velocity projectile introduction and the like. The choice of technique will depend upon the target plant to be transformed. For example, dicotyledonous plants, and certain monocots and gymnosperms, may be transformed by *Agrobacterium* Ti plasmid technology, as described, for example by Bevan, *Nucleic Acids Res.* 12:8711-8721, 1984. Targets for the introduction of the genetic constructs include tissues, such as leaf tissue, disseminated cells, protoplasts, seeds, embryos, meristematic regions, cotyledons, hypocotyls, and the like.

Once the cells are transformed, cells having the genetic construct incorporated in their genome are selected. Transgenic cells may then be cultured in an appropriate medium, using techniques well known in the art. In the case of protoplasts, the cell wall is allowed to reform under appropriate osmotic conditions. In the case of seeds or embryos, an appropriate germination or callus initiation medium is employed. For explants, an appropriate regeneration medium is used. Regeneration of plants is well established for many species. For a review of regeneration of forest trees, *see* Dunstan *et al.*, "Somatic embryogenesis in woody plants," in Thorpe, T.A., ed., *In vitro embryogenesis of plants,* (*Current Plant Science and Biotechnology in Agriculture)*, 20(12):471-540, 1995. Specific protocols for the regeneration of spruce are discussed by Roberts et al. ("Somatic embryogenesis of Spruce," *in* Redenbaugh K., ed., *Synseed: applications of synthetic seed to crop improvement,* CRC Press: Ch.23:427-449, 1993). The resulting transformed plants may be reproduced sexually or asexually, using methods well known in the art, to give successive generations of transgenic plants and practically unlimited amounts of tagged plant-derived products.

The polynucleotides of the present invention may be further employed as non-disruptive tags for marking organisms, particularly microbes. Other organisms may, however, be tagged with the polynucleotides of the present invention, including commercially valuable plants, animals, fish, fungi and yeasts. Genetic constructs comprising polynucleotides of the present invention may be stably introduced into an organism as heterologous, non-functional, non-disruptive tags. It is then possible to identify the origin or source of the organism at a later date by determining the presence or absence of the tag(s) in a sample of material. Detection of the tag(s) may be accomplished using a variety of conventional techniques, and will generally involve the use of nucleic acid probes. Sensitivity in assaying the presence of probe can be usefully increased by using branched oligonucleotides, as described by Horn *et al., Nucleic Acids Res.* 25(23):4842-4849, 1997, enabling detection of as few as 50 DNA molecules in the sample.

Polynucleotides of the present invention may also be used to specifically suppress gene expression by methods that operate post-transcriptionally to block the synthesis of products of targeted genes, such as RNA interference (RNAi), and quelling. Briefly, traditional methods of gene suppression, employing anti-sense RNA or DNA, operate by binding to the reverse sequence of a gene of interest such that binding interferes with subsequent cellular processes and therefore blocks synthesis of the corresponding protein. RNAi also operates on a post-translational level and is sequence specific, but suppresses gene expression far more efficiently. Exemplary methods for controlling or modifying gene expression using RNAi are provided in WO 99/49029 and WO 99/53050. In these methods, post-transcriptional gene silencing is brought about by a sequence-specific RNA degradation process which results in the rapid degradation of transcripts of sequence-related genes. Studies have shown that double-stranded RNA may act as a mediator of sequence-specific gene silencing (see, for example, Montgomery and Fire, *Trends in Genetics*, 14:255-258, 1998). Gene constructs that produce transcripts with self-complementary regions are particularly efficient at gene silencing. A unique feature of this post-transcriptional gene silencing pathway is that silencing is not limited to the cells where it is initiated. The gene-silencing effects may be disseminated to other parts of an organism and even transmitted through the germ line to several generations.

The polynucleotides of the present invention may thus be employed to generate gene silencing constructs and/or gene-specific self-complementary RNA sequences that can be

delivered by conventional art-known methods to cells, such as microbial cells. Within genetic constructs, sense and antisense sequences can be placed in regions flanking an intron sequence in proper splicing orientation with donor and acceptor splicing sites, such that intron sequences are removed during processing of the transcript and sense and antisense sequences, as well as splice junction sequences, bind together to form double-stranded RNA. Alternatively, spacer sequences of various lengths may be employed to separate self-complementary regions of sequence in the construct. During processing of the gene construct transcript, intron sequences are spliced-out, allowing sense and anti-sense sequences, as well as splice junction sequences, to bind forming double-stranded RNA. Select ribonucleases then bind to and cleave the double-stranded RNA, thereby initiating the cascade of events leading to degradation of specific mRNA gene sequences, and silencing specific genes. Alternatively, rather than using a gene construct to express the self-complementary RNA sequences, the gene-specific double-stranded RNA segments are delivered to one or more targeted areas to be internalized into the cell cytoplasm to exert a gene silencing effect. The double-stranded RNA must have sufficient homology to the targeted gene to mediate RNAi and is preferably at least 25 nucleotides in length. Preferably, the double-stranded RNA corresponds specifically to a polynucleotide of the present invention. Gene silencing RNA sequences comprising the polynucleotides of the present invention are useful for creating genetically modified organisms, such as microbes, with desired phenotypes as well as for characterizing genes (for example, in high-throughput screening of sequences), and studying their functions in intact organisms.

In another aspect, the present invention provides methods for using one or more of the inventive polypeptides or polynucleotides to treat disorders in a mammal, such as a human.

In this aspect, the polypeptide or polynucleotide is generally present within a composition, such as a pharmaceutical or immunogenic composition. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Immunogenic compositions may comprise one or more of the above polypeptides and an immunostimulant, such as an adjuvant or a liposome, into which the polypeptide is incorporated.

Alternatively, a composition of the present invention may contain DNA encoding one or more polypeptides described herein, such that the polypeptide is generated *in situ*. In such

compositions, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, and bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminator signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus Calmette-Guerin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other poxvirus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic, or defective, replication competent virus. Techniques for incorporating DNA into such expression systems are well known in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al.*, *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a lipid, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of adjuvants may be employed in the immunogenic compositions of the present invention to non-specifically enhance an immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a non-specific stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *M. tuberculosis*. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories, Detroit,

MI), and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ). Other suitable adjuvants include alum, biodegradable microspheres, monophosphoryl lipid A and Quil A.

Routes and frequency of administration, as well as dosage, vary from individual to individual. In general, the inventive compositions may be administered by injection (*e.g.*, intradermal, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg per kg of host, and preferably from about 100 pg to about 1 μg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 ml to about 2 ml.

The following examples are offered by way of illustration and not by way of limitation.

Example 1

ISOLATION AND CHARACTERIZATION OF DNA SEQUENCES FROM *LACTOBACILLUS RHAMNOSUS* STRAIN HN001

Lactobacillus rhamnosus strain HN001 DNA libraries were constructed and screened as follows.

DNA was prepared in large scale by cultivating the bacteria in 2 x 100 ml cultures with 100 ml MRS broth (Difco Laboratories, Detroit MI) and 1 ml *Lactobacillus* glycerol stock as inoculum, placed into 500 ml culture flasks and incubated at 37 °C for approx. 16 hours with shaking (220 rpm).

The cultures were centrifuged at 3500 rpm for 10 min to pellet the cells. The supernatant was removed and the cell pellet resuspended in 40 ml fresh MRS broth and transferred to clean 500 ml culture flasks. Fresh MRS broth (60 ml) was added to bring the volume back to 100 ml and flasks were incubated for a further 2 hrs at 37°C with shaking (220 rpm). The cells were pelleted by centrifugation (3500 rpm for 10 min) and supernatant removed. Cell pellets were washed twice in 20 ml buffer A (50 mM NaCl, 30 mM Tris pH 8.0, 0.5 mM EDTA).

Cells were resuspended in 2.5 ml buffer B (25% sucrose (w/v), 50 mM Tris pH 8.0, 1 mM EDTA, 20 mg/ml lysozyme, 20 μg/ml mutanolysin) and incubated at 37 °C for 45 min. Equal volumes of EDTA (0.25 M) was added to each tube and allowed to incubate at room temperature for 5 min. 20% SDS (1 ml) solution was added, mixed and incubated at 65 °C for 90 min. 50 μl Proteinase K (Gibco BRL, Gaithersburg, MD) from a stock solution of 20 mg/ml was added and tubes incubated at 65 °C for 15 min.

DNA was extracted with equal volumes of phenol:chloroform:isoamylalcohol (25:24:1). Tubes were centrifuged at 3500 rpm for 40 min. The aqueous phase was removed to clean sterile Oak Ridge centrifuge tubes (30 ml). Crude DNA was precipitated with an equal volume of cold isopropanol and incubated at -20 °C overnight.

After resuspension in 500 μl TE buffer, DNase-free RNase was added to a final concentraion of 100 μg/ml and incubated at 37 °C for 30 min. The incubation was extended for a further 30 min after adding 100 μl Proteinase K from a stock solution of 20 mg/ml. DNA was precipitated with ethanol after a phenol:chloroform:isoamylalcohol (25:24:1) and a chloroform:isoamylalcohol (24:1) extraction and dissolved in 250 μl TE buffer.

DNA was digested with *Sau*3AI at a concentration of 0.004 U/μg in a total volume of 1480 μl, with 996 μl DNA, 138.75 μl 10X REACT 4 buffer and 252.75 μl H_2O. Following incubation for 1 hour at 37 °C, DNA was divided into two tubes. 31 μl 0.5 M EDTA was added to stop the digestion and 17 μl samples were taken for agarose gel analysis. Samples were put into 15 ml Falcon tubes and diluted to 3 ml for loading onto sucrose gradient tubes.

Sucrose gradient size fractionation was conducted as follows. 100 ml of 50% sucrose (w/v) was made in TEN buffer (1M NaCl, 20 mM Tris pH 8.0, 5 mM EDTA) and sterile filtered. Dilutions of 5, 10, 15, 20, 25, 30, 35 and 40% sucrose were prepared and overlaid carefully in Beckman Polyallomer tubes, and kept overnight at 4°C. TEN buffer (4 ml) was loaded onto the gradient, with 3 ml of DNA solution on top. The gradients were centrifuged at 26K for 18 hours at 4°C in a Centricon T-2060 centrifuge using a Kontron TST 28-38 rotor. After deceleration without braking (approx. 1 hour), the gradients were removed and fractions collected using an auto Densi-Flow (Haake-Buchler Instruments). Agarose gel was used to analyze the fractions. The best two pairs of fractions were pooled and diluted to contain less than 10% sucrose. TEN

buffer (4 ml) was added and DNA precipitated with 2 volumes of 100% ice cold ethanol and an overnight incubation at -20°C.

DNA pellets were resuspended in 300 µl TE buffer and re-precipitated for approx. 6 hours at -20 °C after adding 1/10 volume 3 M NaOAC pH 5.2 and 2 volumes of ethanol. DNA was pelleted at top speed in a microcentrifuge for 15 min, washed with 70% ethanol and pelleted again, dried and resuspended in 10 µl TE buffer.

DNA was ligated into dephosphorylated *Bam*HI–digested pBluescript SK II^{+} and dephosphorylated *Bam*HI-digested lambda ZAP Express using standard protocols. Packaging of the DNA was done using Gigapack III Gold packaging extract (Stratagene, La Jolla, CA) following the manufacturer's protocols. Packaged libraries were stored at 4 °C.

Mass excision from the primary packaged phage library was done using XL1-Blue MRF' cells and ExAssist Helper Phage (Stratagene). The excised phagemids were diluted with NZY broth (Gibco BRL, Gaithersburg, MD) and plated out onto LB-kanamycin agar plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) and isopropylthio-beta-galactoside (IPTG). After incubation, single colonies were picked for PCR size determination before the most suitable libraries were selected for sequencing.

Of the colonies picked for DNA minipreps and subsequent sequencing, the large majority contained an insert suitable for sequencing. Positive colonies were cultured in LB broth with kanamycin or ampicillin depending on the vector used, and DNA was purified by means of rapid alkaline lysis minipreps (solutions: Qiagen, Venlo, The Netherlands; clearing plates, Millipore, Bedford, MA). Agarose gels at 1% were used to screen sequencing templates for chromosomal contamination and concentration. Dye terminator sequencing reactions were prepared using a Biomek 2000 robot (Beckman Coulter, Inc., Fullerton, CA) and Hydra 96 (Robbins Scientific, Sunnyvale, CA) for liquid handling. DNA amplification was done in a 9700 PCR machine (Perkin Elmer/Applied Biosystems, Foster City, CA) according to the manufacturer's protocol.

The sequence of the genomic DNA fragments was determined using a Perkin Elmer/Applied Biosystems Division Prism 377 sequencer. The DNA clones were sequenced from the 5' and/or 3' end, and are identified as SEQ ID NOS: 1-80 disclosed herein.

This example not only shows how the sequences were obtained, but also that a bacterium (*E. coli*) can be stably transformed with any desired DNA fragment of the present invention for permanent marking for stable inheritance.

BLASTN Polynucleotide Analysis

The determined DNA sequences were compared to and aligned with known sequences in the public databases. Specifically, the polynucleotides identified in SEQ ID NO: 1-80 were compared to polynucleotides in the EMBL database as of August 12, 2002, using BLASTN algorithm Version 2.0.11 [Jan-20-2000], set to the following running parameters: Unix running command: blastall -p blastn -d embldb -e 10 -G 0 -E 0 -r 1 -v 30 -b 30 -i queryseq –o results. Multiple alignments of redundant sequences were used to build up reliable consensus sequences.

The cDNA sequences of SEQ ID NOS: 1-32 and 34-80 were determined to have less than 60% identity, determined as described above, to sequences in the EMBL database using the computer algorithm BLASTN, as described above. The cDNA sequence of SEQ ID NO: 33 was determined to have less than 90% identity, determined as described above, to sequences in the EMBL database using BLASTN, as described above.

BLASTP Amino Acid Analysis

The polypeptide sequences were compared to sequences in the SwissProt-TrEMBLE protein databases using the computer algorithm BLASTP. Comparisons of amino acid sequences provided in SEQ ID NOS: 81-183 to sequences in the SwissProt-TrEMBLE protein databases (using BLASTP) were made as of August 12, 2002 using BLASTN algorithm Version 2.0.11 [Jan-20-2000], and the following Unix running command: blastall –p blastp –d swissprottrembledb –e 10 –G0 –E0 –v 30 –b 30 –i queryseq –o.

The predicted amino acid sequences of SEQ ID NOS: 84-86, 89, 90, 92, 95, 96, 101-103, 108, 111, 114, 116, 119-122, 124, 125, 130, 134-136, 140, 146, 147, 152, 156, 159, 162, 164, 166, 168, 175 and 183 were determined to have less than 50% identity, determined as described above, to sequences in the SWISSPROT-TrEMBLE database using the BLASTP computer algorithm as described above. The predicted amino acid sequences of SEQ ID NOS: 81-83, 88, 91, 93, 94, 97-100, 104-107, 109, 110, 112, 113, 115, 123, 127-129, 131-133, 137, 138, 141-145,

148-151, 153-155, 157, 158, 160, 161, 163, 165, 167, 169-173 and 180-182 were determined to have less than 75% identity, determined as described above, to sequences in the SWISSPROT-TrEMBLE database using the computer algorithm BLASTP, as described above. The predicted amino acid sequences of SEQ ID NOS: 87, 139 and 176-179 were determined to have less than 90% identity, determined as described above, to sequences in the SWISSPROT-TrEMBLE database using the computer algorithm BLASTP, as described above. The predicted amino acid sequences of SEQ ID NOS: 117, 118 and 126 were determined to have less than 98% identity, determined as described above, to sequences in the SWISSPROT-TrEMBLE database using the computer algorithm BLASTP, as described above.

BLASTX Polynucleotide Analysis

The isolated cDNA sequences were compared to sequences in the SwissProt-TrEMBLE protein databases using the computer algorithm BLASTX. Comparisons of DNA sequences provided in SEQ ID NOS: 1-80, to sequences in the SwissProt-TrEMBLE database (using BLASTX) were made as of August 12, 2002 using BLAST algorithm Version 2.0.11 [Jan-20-2000], and the following Unix running command: blastall –p blastx –d swissprottrembleldb –e 10 –G0 –E0 –v 30 –b 30 –i queryseq –o.

The cDNA sequences of SEQ ID NOS: 1-14, 16-49, 52-58, 60-72, 74-78 and 80 were determined to have less than 50% identity, determined as described above, to sequences in the SWISSPROT-TrEMBLE database using the computer algorithm BLASTX, as described above. The cDNA sequences of SEQ ID NOS: 15, 50, 51, 59, 73 and 79 were determined to have less than 75% identity, determined as described above, to sequences in the SWISSPROT-TrEMBLE database using BLASTX, as described above.

Based on similarity to known sequences, the isolated polynucleotides of the present invention identified as SEQ ID NOS: 1-80 were putatively identified as encoding polypeptides having similarity to the polypeptides shown above in Table 1. The amino acid sequences encoded by the DNA sequences of SEQ ID NO: 1-80 are provided in SEQ ID NO: 81-183, respectively.

Several of the sequences provided in SEQ ID NO: 1-80 were found to be full-length and to contain open reading frames (ORFs). These full-length sequences, the location of ORFs (by

nucleotide position) contained within these sequences, and the corresponding amino acid sequences are provided in Table 2 below.

TABLE 2

Polynucleotide SEQ ID NO:	ORF	Polypeptide SEQ ID NO:
1	5831-7288	81
2	4395-5630	82
3	1445-2791	83
4	316-1413	84
5	1392-2444	85
6	1-1083	86
7	2881-4071	87
8	1859-3295	88
9	265-1023	89
10	1160-2005	90
11	2324-3604	91
11	548-1696	92
12	1102-2358	93
12	188-1020	94
13	140-1138	95
14	5612-6413	96
15	84-2276	97
16	1130-2275	98
17	1644-2645	99
18	500-2404	100
19	110-1153	101
20	167-718	102
21	1-3669	103
22	2327-4951	104
23	522-1694	105
24	973-2928	106
25	133-1296	107
26	1938-3497	108
27	69-716	109
28	125-1054	110
29	84-3377	111
30	555-887	112
30	226-558	113
31	77-862	114
32	3135-4673	115
32	6384-7877	116

Polynucleotide SEQ ID NO:	ORF	Polypeptide SEQ ID NO:
33	412-828	117
33	863-1663	118
34	1642-2682	119
35	814-2037	120
35	3510-5084	121
36	101-1222	122
37	113-760	123
38	1-477	124
39	12447-13400	125
40	1186-2439	126
41	7973-8812	127
41	6950-7924	128
41	1925-2773	129
41	3916-4956	130
41	1023-1895	131
41	8822-10489	132
42	102-860	133
43	3759-4343	134
44	622-1113	135
45	1129-1722	136
46	1216-2439	137
46	2345-3835	138
47	6155-8361	139
48	550-1176	140
49	455-901	141
51	31-1179	143
52	766-2142	144
53	2336-3817	145
54	7567-9057	146
55	3713-4447	147
56	426-1625	148
57	138-851	149
58	3066-4769	150
59	31-1188	151
60	132-1328	152
61	94-534	153
62	652-1998	154
63	1033-1905	155
63	1902-2789	156
64	951-1646	157
64	1824-3227	158
65	179-1030	159

Polynucleotide SEQ ID NO:	ORF	Polypeptide SEQ ID NO:
66	1244-2425	160
67	6885-7589	161
68	333-1121	162
69	2638-4251	163
69	1312-2622	164
70	1587-2441	165
71	4642-6489	166
71	7554-8516	167
71	6625-7536	168
71	8518-9469	169
72	2344-3732	170
72	3755-5674	171
73	102-1874	172
74	2993-4429	173
75	1008-1499	174
76	3565-5967	175
77	759-1708	176
77	1488-2837	177
77	3295-4074	178
77	4071-5096	179
77	2692-3291	180
78	165-842	181
79	66-2291	182
80	269-1006	183

Example 2

ISOLATION AND CHARACTERIZATION OF PURINE NUCLEOSIDE PHOSPHORYLASE FROM *L. RHAMNOSUS* STRAIN HN001

The full-length polynucleotide sequence of the deoD purine nucleoside phosphorylase gene *AQ1* from *L. rhamnosus* HN001 is given in SEQ ID NO: 78 and shown in Fig. 1 (with ATG initiation and translation stop codons boxed). The polypeptide sequence of *AQ1* is given in SEQ ID NO: 181 and shown in Fig. 2.

A 634 bp internal *AQ1* fragment was amplified by PCR using standard laboratory protocols. The nucleotide sequences of the oligonucleotide primers are given in SEQ ID NOS: 184 and 185. The fragments were cloned into the pBEry1 vector cut with *Sma*I. The 3.6 kb

pBEry1 vector was constructed using the replicon and multiple cloning site (MCS) from the phagemid pBlueScript (pBS-SK+) (Stratagene, La Jolla CA, USA). The ampicillin resistance gene in pBS-SK+ was removed by digestion with *Rca*I (Roche, Auckland, New Zealand), and the 1,953 bp fragment containing the ColE1 origin and multiple cloning site purified and treated with Klenow enzyme (Roche) to give a blunt-ended fragment. A gene encoding resistance to erythromycin (Em) was isolated on a 1.6 kb fragment obtained after cutting pVA891 (Macrina *et al.*, *Gene* 25:145-50, 1983) with *Cla*I and *Hin*dIII and treatment with Klenow to give blunt ends. The 1.6 kb Em fragment was ligated to the 1,953 bp pBS-SK+ fragment, transformed into *E. coli* TG1 (Gibson TJ, *Studies on the Epstein-Barr virus genome*. Ph.D. Thesis, University of Cambridge, Cambridge, England, 1984), and plated on LB agar plates containing 200 μg/ml Em. Maintenance of α-complementation for blue/white color selection of recombinant pBEry1 clones was confirmed by growing *E. coli* colonies on agar plates containing IPTG and X-gal.

The resulting pBEry1 construct encoding the HN001 deoD purine nucleoside phosphorylase *AQ1* gene was transformed into competent HN001 cells and grown anaerobically for 48 hrs at 37 °C on MRS lactobacilli agar (Difco, Detroit MI) containing 2.5 μg/ml Em. Em-resistant HN001 were checked for integration of the plasmid construct into the *deoD* gene by PCR using vector-specific (T3 or T7) and *AQ1* internal fragment-specific primers.

Colonies giving PCR patterns consistent with the insertional inactivation of the endogenous HN001 deoD purine nucleoside phosphorylase *AQ1* gene were assessed for increased resistance to UV irradiation. Briefly, single colonies of wild-type of *AQ1*-knockout HN001 strains were inoculated into 5 ml MRS, incubated aerobically overnight at 37 °C, and 0.1 ml then used to inoculate a 10 ml MRS culture. Cultures were grown to log phase (i.e. an OD_{600} of approximately 0.3) at 37 °C, and then 5 ml of culture taken and cells collected by centrifugation. Cells were resuspended in 1 ml normal saline (0.9% NaCl), and 20 μl aliquots placed on sterile petri dishes. Petri dishes were then placed uncovered and inverted onto a standard laboratory transilluminator and exposed to UV light for 0, 20 or 30 seconds. Samples were transferred to 1 ml MRS media and grown for 2 hours at 37 °C in the dark. Following culture, samples were appropriately diluted and duplicate samples plated onto MRS plates, incubated anaerobically for 48 hours at 37 °C and colonies counted.

Fig. 3 shows the results of UV light exposure assay measuring relative viability in response to increasing doses of UV light for *AQ1*⁻ HN001 strain (♦) and wild-type HN001 (■). Results indicate that the *AQ1*⁻ HN001 mutant strain showed enhanced survival to exposure to UV light compared to wild-type HN001. UV light exposure of 20 seconds appeared to have no effect on *AQ1*⁻ viability while the viability of wild-type HN001 cells had dropped to 34.7%. After 30 seconds UV light exposure, 86.9% of *AQ1*⁻ cells survived compared to only 27.9% for wild type. Therefore, removal of *AQ1* gene expression led to enhanced survival of UV light exposure, indicating that *AQ1* encodes the HN001 deoD purine nucleoside phosphorylase.

Purine nucleoside phosphorylase (EC 2.4.2.1) is involved in the purine biosynthesis and salvage pathways. Its role in maintaining intracellular guanosine pools suggests that it may be involved in resistance to a number of stress conditions including UV light exposure, as well as high salt, pH and temperature (Duwat *et al, Int J Food Microbiol.* 55:83-6, 2000). Applications for HN001 purine nucleoside phosphorylase AQ1 include:

- methods of enhanced survival of industrial processes;
- improved colonization of human intestinal environment; and
- improved survival of multiple stress conditions.

Example 3

ISOLATION AND CHARACTERIZATION OF GTP PYROPHOSPHOKINASE FROM *L. RHAMNOSUS* HN001

The full-length polynucleotide sequence of the relA GTP pyrophosphokinase gene *AM1* from *L. rhamnosus* HN001 is given in SEQ ID NO: 79 and shown in Fig. 4 (with ATG initiation and translation stop codons boxed). The polypeptide sequence of AM1 is given in SEQ ID NO: 182 and shown in Fig. 5.

A 798 bp internal *AM1* fragment was amplified by PCR using standard laboratory protocols. The nucleotide sequences of the oligonucleotide primers are given in SEQ ID NOS: 186 and 187. The fragments were cloned into the pBEry1 vector cut with *Sma*I, as described in Example 2. The resulting pBEry1 construct encoding the HN001 relA GTP pyrophosphokinase *AM1* gene was transformed into competent HN001 cells and grown anaerobically for 48 hrs at 37

°C on MRS lactobacilli agar (Difco, Detroit MI) containing 2.5 μg/ml Em. Em-resistant HN001 were checked for integration of the plasmid construct into the *relA* gene by PCR using vector-specific (T3 or T7) and AM1 internal fragment-specific primers.

Colonies giving PCR patterns consistent with the insertional inactivation of the endogenous HN001 relA GTP pyrophosphokinase *AM1* gene, were assessed for increased resistance to UV irradiation as described in Example 2.

Fig. 6 shows the results of a UV light exposure assay measuring relative viability in response to increasing doses of UV light in *AM1*⁻ HN001 (♦) and wild-type HN001 (■) strains. Results indicate that the *AM1*⁻ HN001 mutant strain showed enhanced survival to exposure to UV light compared to wild-type HN001. UV light exposure of 20 seconds appeared to have little effect on *AM1*⁻ cell viability (91.7%) while the viability of wild-type HN001 cells had dropped to 34.7%. After 30 seconds UV light exposure, 61.1% of *AM1*⁻ cells survived compared to only 27.9% for wild type. Therefore, removal of *AM1* gene expression led to enhanced survival of UV light exposure, indicating that *AM1* encodes the HN001 relA GTP pyrophosphokinase.

GTP pyrophosphokinase or (EC 2.7.6.5) produces guanosine 3'-diphosphate 5'-triphosphate, a marker of the "stringent response", a regulatory state induced in bacteria by nutrient starvation and other environmental stresses (reviewed in Chatterji and Ojha, *Curr Opin Microbiol.* 4:160-5, 2001). Studies have indicated that suppression of GTP pyrophosphokinase *relA* gene expression improved the resistance to a number of stress conditions including UV light exposure, as well as high salt, pH and temperature, in *Lactococcus lactis* (Duwat *et al, Int J. Food Microbiol.* 55:83-6, 2000). Applications for HN001 GTP pyrophosphokinase AM1 include:

- methods of enhanced survival of industrial processes;
- improved colonization of human intestinal environment; and
- improved survival of *Lactobacilli* to multiple stress conditions.

SEQ ID NOS: 1-187 are set out in the attached Sequence Listing. The codes for nucleotide sequences used in the attached Sequence Listing, including the symbol "n," conform to WIPO Standard ST.25 (1998), Appendix 2, Table 1.

All references cited herein, including patent references and non-patent publications, are hereby incorporated by reference in their entireties.

While in the foregoing specification this invention has been described in relation to certain preferred embodiments, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.